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Biochemistry

For Medical Students
Part II



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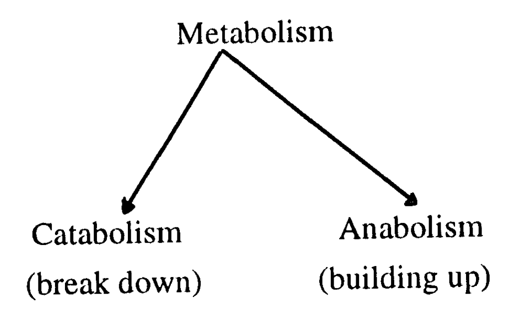
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CHAPTER I

METABOLISM

Metabolism is concerned with all the chemical changes that occur inside the body to get energy or to form biologically active compounds from the food materials . Metabolism also involves the changes that the body produce in the xenobiotics to reduce their toxicity and to help their excretion :



Methods for studying the metabolism

1. Whole animal experiment :
   The tested substance is introduced to the animal body and the metabolites are collected.
2. Removal of an organ to study the functional and metabolic defects.
3. Isolated organ in sito.
4. Tissue slices "in vitro".
5. Tissue homogenate and its fractions.
6. Isolation of specific enzyme.
7. The use of radiolabeled " radioactive isotopes" to trace the uptake and metabolites of certain substances in the cell.

ISOTOPES

The atom is composed of :

(A) Nucleus that contain neutrons and protons (+) .

(B) Electrons that rotate around the nucleus in specific shells or orbits. Each orbit has a specific energy level, that increase as we go away from the nucleus. Electrons are very light and carry (-ve) charge. Their number is equal to the number of the protons .

Atomic Number : the number of protons that is equal to the number of electrons. This number determines the valency and chemical properties.

Atomic mass = " Mass Number ":

It is the number of protons + neutrons. It determines the mass and the Physical properties of an element.

The weight of an atom is equal to protons + Neutrons + electron (negligible)

Definition of isotopes

Isotopes are atoms having the same atomic number but differing in the mass number. They have therefore, the same chemical properties and differs in the physical properties.

e.g. isotopes of hydrogen ^{1}H, (ordinary hydrogen) ^{2}H, (Deuterium) ^{3}H (Tritium)

Types of isotopes :

(A) Stable isotopes " Not radioactive "

They have stable nucleus .

As ^{1}H , ^{2}H and ^{12}C, ^{13}C

(B) Radioactive isotope

Their nucleus is unstable and the atoms decompose " disintegrate" and emit one or more of the following radiations.

1- α -particles which are positively charged nuclei of helium (^{4}He)

2. ß-particle which are negatively charged electrons.

3-γ -Rays which are electromagnetic radiations of very short wave length.

Examples : ^{3}H, ^{14}C, ^{125}I, ^{131}I.

Existence of Isotopes:

Isotopes may be present in nature or they are prepared in cyclotrons.

Uses of isotopes in Medicine :

Isotopes are used in the medical field for diagnosis , treatment and studying the metabolism. Examples are given below .

1. Diagnosis of some diseases of the thyroid glands as malignancy of the thyroid. The ^{131}I is introduced into the patients body and its uptake by the thyroid gland cells can be traced to determine the activity of the gland in its different areas. Malignant areas are hyperactive.
2. Treatment of malignant diseases " Radiotherapy" Radioactive iodine " ^{131}I " can be used for treatment of thyroid tumors to kill the malignant cells. Radioactive cobalt "^{60}Co" or phosphorus "^{32}P" can be used for treatment of cancers.
3. Studying the metabolism can be done by tracing radiolabelled isotopes during the biochemical transformation of different compounds.

BIOLOGICAL OXIDATION AND THE RESPIRATORY CHAIN

Energy is derived from oxidation of carbohydrates, proteins and lipids. The liberated energy is partially converted into ATP and partially into heat energy.

Oxidation means removal of hydrogen or electrons or addition of oxygen. Reduction is the opposite of oxidation. Oxidation and reduction reactions occurs at the same time and are therefore given the name redox reactions.

The enzymes that catalyse the oxidation-reduction reactions are called oxido-reductases.

The oxidoreductases include :

1. Oxygenases which include mono or dioxygenase. They add either O or O_2 respectively.

2. Oxidases as cytochrom oxidase which uses oxygen atom as acceptor to the hydrogen and form water. Amino acid oxidases (D and L) use O_2 as acceptor to the removed hydrogen and form H_2O_2 " hydrogen peroxide" . Xanthine oxidase belongs to these oxidases).

3. Dehydrogenases. they remove the hydrogen from the substrate to be carried on NAD or FAD coenzymes . The reduced forms of these coenzymes ($NADH+H^+$ and $FADH_2$) are then oxidized in the respiratory chain in the mitochondria.

4. Hydroperoxidases :

They deal with hydrogen peroxide (H_2O_2) produced by D and L-amino acid oxidases and xanthine oxidase activity.H_2O_2 is unstable and dissociates into H_2O and atomic oxygen O.

H_2O_2 --------> $H_2O + O$

The atomic oxygen is very reactive and has a destructive effect

on the cell membranes . Most tissues have peroxidases and catalases that can break down H_2O_2. Example of the peroxidases is the glutathion peroxidase which contain selenium.

$$2\,GSH + H_2O_2 \text{ as } \xrightarrow[\text{Selenium (Se)}]{\text{Geutathioneperoxidase}} GS\text{-}SG + H_2O$$

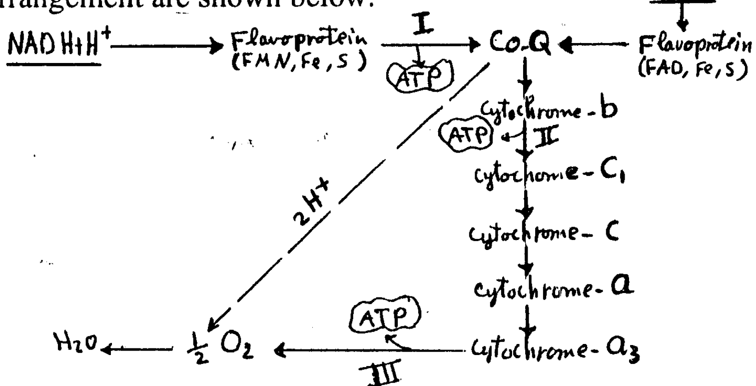
The catalase uses H_2O_2 donor and acceptor

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

The Respiratory chain:

The oxidation of the food materials as glucose, fatty acids and amino acids is mainly done by removal of hydrogen to be carried by the coenzymes NAD and FAD. The reduced coenzymes $NADH+H^+$ and $FADH_2$ deliver their hydrogen as H^+ and electrons $(H+,e^-)$ to O_2 in steps through the components of the respiratory chain. The movement of the electrons creates energy that is partly used for phosphorylation of ADP to form ATP and partially as heat.

The components of the respiratory chain are located within the inner mitochondrial membrane. They are arranged in an increasing redox potential to guarantee the rapid movement of the H^+ and electrons (e) . The redox potrential is the electron affinity. Hydrogen has the least while oxygen has the highest affinity to electrons. The components and their arrangement are shown below.



*The $NADH+H^+$ and $FADH_2$ are the reducing equivalents that are formed during oxidation of the glucose, fatty acids or amino acids.

* The Flavoproteins are dehydrogenases. Some flavoproteins contain FMN, iron that shuttles between Fe^{++} (ferrous) and Fe^{+++} (ferric) and contain sulphur. These flavoproteins transfer $2H^+$ and 2e from $NADH+H^+$ to CO-Q. Other flavoproteins contain FAD, Fe and S and transfer $2H^+$ and 2 e from $FADH_2$ to CO-Q.

The Co-Q " ubiquinone" is similar to vitaminK in structure. both have quinone ring that can accept $2H^+$ and 2e to change into quinol ring.

quinone ⇌ (2H⁺e⁻ / 2He⁻) quinol

*The cytochromes are arranged in the sequence b, c_1, c, a, a_3 they are proteins which have a haem prosthetic group similar to that of haemoglobin. The iron atom in haem alternates between the ferrous (Fe^{++}) and ferric (Fe^{+++}) states by accepting or donating electron (e).

$$Fe^{++} \underset{e^-}{\overset{e^-}{\rightleftharpoons}} Fe^{+++}$$

Thus each cytochrome can accept and propagate the electrons. Cytochrome b contain sulphur in addition, and cyt. a3 contains copper.

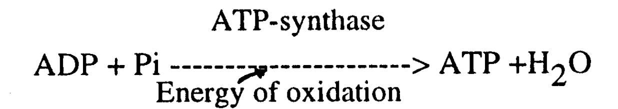
*At the final stage of the respiratory chain, the electrons are transferred to $1/2O_2$ and the hydrogen ions 2H+ are conducted directly from CO-Q to the 1/2 O_2 forming water molecule H_2O

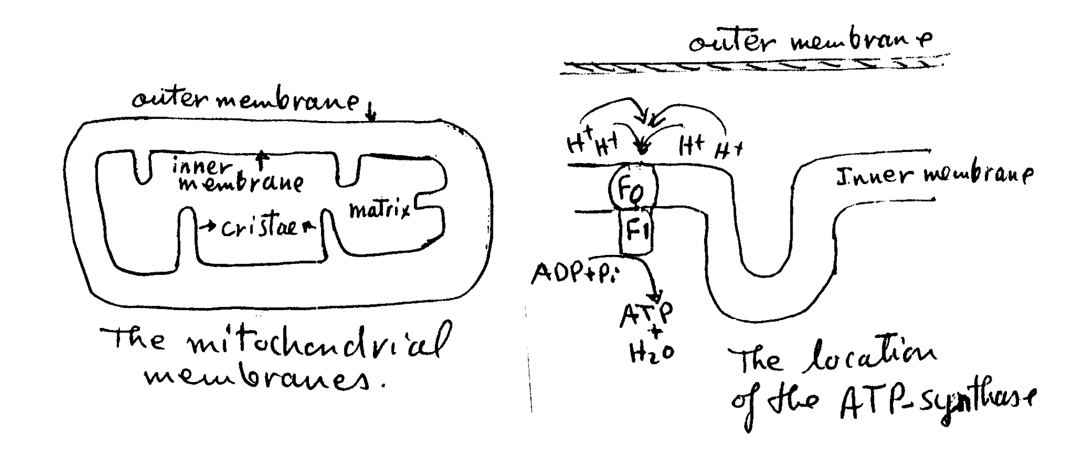
The electron flow through the rspiratory chain causes a release of energy. At the level between flavoprotein FMN and Co-Q (Site I) the released energy is sufficient to phosphorylate ADP to form ATP . A second ATP is formed at site II and a third one at site III in the chain.

The molecular mechanism by which the electron flow is coupled to the phosphorylation of ADP is not definitely known. The chemio-osmotic theory is the most acceptable one.

This theory explains the mechanism of coupling the energy of oxidation with phosphorylation of ADP. This energy causes the hydrogen ions (H^+) to be pumped to the outside of the inner mitochondrial membrane. This membrane is impermeable to ions especially hydrogen ions (H^+) or protons which accumulate outside this inner membrane, creating an electro-chemical potential difference across the membrane. The accumulated protons return back to the inside of the inner membrane through the protein that forms the ATP-synthase enzyme within the membrane at the three sites shown before .

ATP-synthase is formed of 2 subunits namely "FO" which is embeded within the inner membrane and "Fl" subunit which projects into the matrix of the mitochondria. The passage of protons (H^+) from outside to the inside of the inner membrane through the (FO-Fl) subunits of ATP-synthase causes formation of ATP from ADP and inorganic phosphate (Pi).





The mitochondrial membranes.

The location of the ATP-synthase

Inhibitors of electron transport through the respiratory chain

The inhibitors of the respiratory chain arrest the respiration by blocking the flow of electrons at three sites in the respiratory chain.

Site I : between complex I or the flavoprotein- FMN and Co-Q . They include :-

(a) Barbiturates which are used as drugs for sedation and hypnosis.

(b) Rotenone which is an insecticide and fish poison.

(c) Pericidin A " Antibiotics"

High dosages of the above listed agents are fatal in vivo.

Site II : between cytochrome b and c_1 .

The inhibitors at this site include :

(a) BAL " British Anti-Lewisite ". it is an antiarsenical drug.

(b) Antimycin-A " antibiotic".

Site III : At the cytochrome oxidase level. The inhibitors at this site bind to the cytochrome oxidase " cyt. a_3" and block its activity . They include :

(a) Hydrogen sulphide "H_2S"

(b) Carbon monoxide " CO"

(c) Cyanides "CN"

These three inhibitors are called the classic poisons and can totally stop respiration.

Inhibitors of oxidative phosphorylation" Uncouplers"

The uncouplers dissociate oxidation in the respiratory chain from phosphorylation of ADP. Uncouplers, thus decrease the formation of ATP and most of the generated energy will be liberated as heat. The uncouplers increase the permeability of the inner mitochondrial membrane to protons and let the proton pass through the membrane

away from the ATP-synthase, thus oxidation proceed without phosphorylation of ADP.

The uncouplers include :

1. Calcium injection.

Calcium injections as during treatment of tetany causes sensation of increased body temperature.

2. progesterone hormone.

Progesterone is secreted from the corpus lutium in the ovary just after ovulation leading to elevation of the females body temperature. This phenomenon is used as a test of the ovulation in the females.

3. Thyroxine (T4)

Thyroxine in high dose as in cases of hyperthyroidism causes elevation of body temperature and increase oxygen consumption.

4. Dinitrophenol. It is drug used for weight reduction. it is no longer used due to its toxicity.

5. Oligomycin.

it is a fungicidal drug used for the treatment of fungal infections.

6. Bilirubin.

The bilirubin level in the blood is increased in cases of jaundice. It's high level in the blood is accompanied by elevation of body temperature.

Carbohydrate Metabolism

Digestion and absorption:

Carbohydrates in our food include

1. Starch 2. Glycogen 3. Cellulose	**Polysaccharides**
4 . Sucrose 5. Lactose 6. Maltose	**disaccharides**
7. Glucose 8. Fructose 9. Galactose 10. Pentose	**Monosacchrides**

Only , polysaccharides and disacharides need digestion .

(A) Digestion in the mouth.

Salivary amylase

Starch. or glycogen ---------------> maltose + dextrins

(B) Digestion in the stomach.

There is no digestion of carbohydrates in the stomach . The high acidity in the stomach stops the activity of the salivary amylase.

(C) Digestion of carbohydrates in upper small intestine.

(1) Starch or or dextrin --Pancreatic amylase--> maltose + isomaltose.

(2) Maltose --Maltase--> glucose + glucose

Sucrose --Sucrase--> glucose + fructose

Lactose --Lactase--> glucose + galactose

These disaccharidases are present at the brush border of the intestinal mucosa cells.

Absorption:

Monosaccharides are the only absorbable forms of sugars.

*Glucose and galactose are absorbed by the same carrier protein. The absorption is active " need energy", sodium dependent .

* Fractose and pentoses are mostly absorbed by passive diffusion.

* The absorbed sugars passes through the intestinal mucosa to reach the portal blood.

Thus all the monosaccharides " glucose, fructose and galactose" are absorbed to the portal blood to reach the liver. In the liver, galactose and fructose change into glucose. Glucose passes from the liver through the hepatic vein to the systemic blood. Therefore, glucose is the main sugar of the systemic blood.

Normal level of blood sugar:

*Fasting level " 12 hours fasting"

70-110 mg/dl. deciliter = 1/10 liter

(70-110 mg/100 ml of blood.)

* One hour post prandial " 1 h PP"

(after meal)

up to 150 mg /dl

* Two hours post prandial " 2 h PP"

70 - 110 mg/dl.

The glucose is utilized in the body for :-

1. Oxidation to get energy .
2. Storage as Glycogen in all tissues especial in liver and muscles.
3. Change into other biologically important materials as :

   glucose ------------> Ribose and deoxyribose.

   glucose ------------> glucuronic acid

   glucose ------------> galactose in the lacting mammary gland

   glucose ------------> fructose in the seminal vesicle.
4. Glucose can change into fats in the adipose tissue. Excess carbohydrates in food is a direct cause of obesity.

Glucose oxidation:

Glucose oxidation occurs at three levels .

1. Glucose through Glycolysis produces pyruvic acid or lactic acid in the cytoplasm of all cells.
2. Pyruvic acid is changed to acetyl COA

   pyruvic ----------------> Acetyl COA

   by oxidative decarboxylation. This process occurs in the mitochondria.
3. Acetyl COA is used up in the Kreb's cycle" Tricarboxylic acid cycle".

Glycolysis

Definition:

Glycolysis is oxidation of glucose to get energy. The end product may be either :

(a) 2 Pyruvate in the presence of sufficient oxygen and mitochondria "aerobic glycolysis"

(b) 2 lactate in the lack of oxygen as in the contracting muscle or in absence of mitochondria as in RBC,s. "anaerobic glycolysis".

Steps : All the steps occur in the cytoplasm

1-Glucose uptake from the blood into the cell. This step depends on insulin hormone in muscle and adipose tissue cells. Deficiency of Insulin as in Diabetes Mellitus decreases glucose uptake and causes hyperglycemia.

2-Inside the cell, glucose is changed into glucose – 6 – phosphate .

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{Glucokinase " in liver"}]{\text{Hexokinase "in all tissues"}} \text{Glucose–6- phosphate} + \text{ADP}$$

3-Through many enzymatic steps the glucose –6– P is split into two molecules of glyceraldehyde –3– P with consumption of a second ATP.

4- The two molecules of glyceraldehyde – 3 – P are oxidized by enzymes into 2 pyruvic acids with production of 2 $NADH+H^+$ and 4 molecules of ATP.

5-In anaerobic conditions the $2NADH+H^+$ are reoxidized into 2 NAD by changing the two pyruvic acids into 2 lactic acids.

$$\text{Pyruvic} + NADH+H^+ \xrightarrow{\text{LDH}} \text{Lactic} + NAD$$

- In aerobic glycolysis the 2 $NADH+H^+$ are reoxidized in the respiratory chain with production of 3 ATP for each $NADH+H^+$.

Energy gain " yield " of glycolysis

1- In anaerobic glycolysis.
- 2 ATP are consumed in glycolysis
- 4 ATP are produced . The net is 2 ATP.

2- In aerobic glycolysis.
- 2 ATP are consumed
- 4 ATP are produced .
- 6 or 4 ATP are produced from oxidation of 2 $NADH+H^+$ in the respiratory chain.
- The net is 4 + (6 or 4) _ 2 = 8 or 6 ATP .

Oxidative decraboxylation of pyruvate

Inside the mitochondria, pyruvic acid is oxidized to acetyle-COA and $NADH+H^+$ is produced. 5 vitamins are necessary to complete this reaction. These vitamins act as coenzymes or part of coenzymes required for the enzyme complex pyruvate chehydrogenase. The oxidative decarbonxylation reaction is summarized as

$$\text{Pyruvic} + NAD \xrightarrow[\text{TPP, Lipoic , FAD , COA-SH}]{\text{Pyruvate dehydrogenase complex}} CO2 + \text{Acetyl-COA}+NADH+H^+$$

The 5 vitamins used in this reaction are:

1- Vitamin – B_1 " Thiamine" as Thiamine pyrophosphate "TPP".

2- Lipoic acid "one of B-Complex Vitamins"

3- Vitamin-B_2 "Riboflavine" as flavine Adenine Dinucleatide "FAD".

4- Pantothenic acid as part of coenzyme – A "COA-SH".

5- Nicotinic acid "Niacin" as part of Nicotinamide Adenine Dinucleatide "NAD".

Deficiency of these vitamins, therefore, inhibits the process of energy production from carbohydrates.

Kreb's cycle "Citric Acid Cycle Tricarboxylic Acid Cycle"

Acetyle–COA condenses with oxaloacetic acid inside the mitochondria to form citric acid that enter in series of reactions that end with formation of oxaloacetic acid. Through this cycle, Acetyle COA is oxidized to CO_2 , H_2O and energy as ATP and $NADH+H^+$ that is oxidized in the respiratory chain to form more ATP.

Energy Yield of Kreb's cycle

- 12 ATP are produced in each cycle.
- Since one glucose molecule produce 2 pyruvic acid molecules that gives two acetyl COA and two $NADH+H^+$ "equivalent to 2 × 3 ATP" , the energy, yield from complete oxidation of glucose is calculated as follows:

- In aerobic glycolysis , one glucose produce 6 or 8 ATP and 2 pyruvic acid.
- In oxidative decarboxylution of the 2 pyruvic acids two $NADH+H^+$ are produced which gives $2 \times 3 = 6$ ATP in the respiratory chain and two acetyl COA .
- In Kreb's cycle, the 2 acetyl-COA yield $2 \times 12 = 24$ ATP .
- The net energy yield from complete oxidation of glucose molecule is:
  $(6 \text{ or } 8) + 6 + 24 = 36 \text{ or } 38$ ATP.

Pentose Phosphate Pathway
" Hexose Monophosphate Pathway"

Definition: It is alternative pathway for glucose oxidation which does not produce ATP.

Importance : This pathway utilizes glucose to produce two important compounds which are :

(a)Ribose from glucose. This is the main source of ribose which is utilized for synthesis of the nucleotides that are used as building units of nucleic acids "RNA & DNA" , as part of many coenzymes and as energy carriers "eg. ATP".

(b) NADPH+H^+ "reduced coenzyme II"

This reduced coenzyme II is used by many tissue as a donor for Hydrogen which is necessary for synthesis of fatty acids, cholesterol, steroid hormones and for the regeneration of the active reduced glutathione "G.SH".

Steps :

The Pentose phosphate pathway occurs in 2 phases:

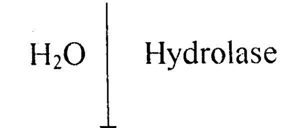
(a) Oxidative phase "Non reversible

(b) Non oxidative phase "reversible"

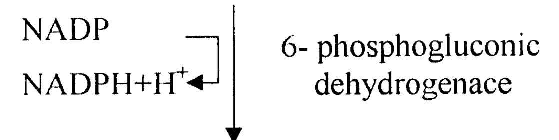
3 x Glucose – 6 – phosphate + NADP

↓ Glucose – 6 – phosphate Dehydrogenase

3 x 6- phosphogulcono- lactone + NADPH+H^+



3 x 6- phosphogluconic



3 x Ribulose – 5 – phosphate + $3CO_2$

Isomerase ↕ epimerase

Ribose –5-P | Xylulose-5-P | Xylulose –5-P

Transketolase (TPP)
Transaldolase

Transaldolaue
TPP

Fructose-6-P | erythrose-4-P →

Fructose –6-P + Glyceraldehyde –3-P

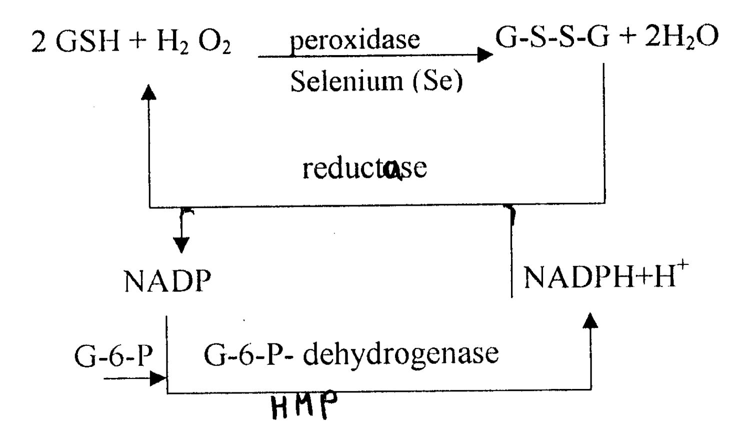
Glucose –6- phosphate dehydrogenase deficiency "Favism"

Definition:

- It is a hereditary disease caused by deficiency of the G-6-P dehydrogenate enzyme of the pentose phosphate pathway especially in RBC,S.

Effects

- The production of NADPH+H^+ is greatly reduced. The deficiency of NADPH+H^+ inhibits the synthesis of fats in the cell membrane and inhibits the formation of the active reduced glutathione "GSH" which removes the toxic effect of hydrogen peroxide "$H_2 O_2$".
- The increase in the level of the toxic $H_2 O_2$ and the decrease in the synthesis of fats in RBC,S membrane lead to hemolysis "Hemalytic anemia".



- The crises of hemolysis occurs when the patient receives substances that are known to have oxidant effect and which needs larger amounts of NADPH+H^+ as Aspirin, primaquine, sulphonamide and fava beans.

Gluconeogenesis

Definition: Synthesis of glucose from non-carbohydrate sources as

1- Lactic acid produced during muscle contraction.

2- Glycerol produced by lipolysis of triglycerides during fasting.

3- Glucogenic amino acids as alanine.

Site: Liver and Kidney

Steps: The pathway of gluconeogenesis is mainly the reversal of the glycolytic pathway "glycolysis".

Importance of gluconeogenesis:

1- Gluconeogenesis supplies the body with glucose during fasting and starvation especially when the liver glycogen is consumed.

2- It removes the lactic acid and glycerol from the blood.

Control: Insulin inhibits gluconeogensis while anti-insulin hormones especially cortisone stimulate it. Fasting stimulates while feeding inhibits it.

Glycogen Metabolism

- Glycogen is the storage from of glucose in animal cells especially in the liver and muscle cells.
- **Glycogen synthesis "Glycogenesis"**

Steps:

Glycogen synthesis occurs by addition of glucose units from Uridine diphospho - glucose (UDP-glucose) to a glycogen primer by the enzyme glycogen synthase. Branching enzyme forms the branches in glycogen molecule.

UDP- glucose + Glycogen primer

↓ glycogen synthase

straight chain of glycogen

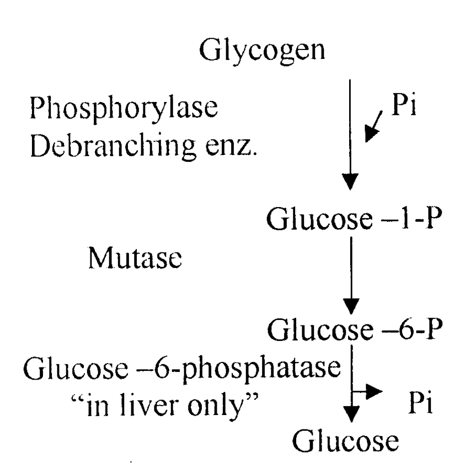
↓ Branching enzyme

Glycogen molecule with branched chain.

- Glycogen synthesis occurs after ingestion of carbohydrate and utilizes about 20% of the carbohydrate in a normal meal. It is stimulated by Insulin and inhibited by anti-insulin hormones.

- **<u>Glycogen breakdown "Glycogenolysis"</u>**

The breakdown of glycogen into glucose in the liver and into glucose –6- phosphate in the muscle starts to occur after the onset of fasting "2 hours after meal". The enzyme needed for glycogenolysis are glycogen phosphorylases and the debranching enzyme.



- Glycogenolysis is stimulated by fasting. During fasting the liver glycogen is changed to glucose to supply the rest of the body cells, and the muscle glycogen is changed to glucose –6-P to be utilized by the muscle itself during contraction. Insulin inhibits while anti-insulin hormones stimulate glycogenlysis.

BLOOD GLUCOSE

The normal fasting blood glucose level is 70-110 mg/dl afte 8-12 hours fasting. This level rises up to 150 mg/dl after meal and returns back to the fasting level two hours after meal, then the blood glucose level remains constant at the fasting level until the next meal.

During fasting which starts two hours after meal(2 hours post prandial), the sources of blood glucose are :

(A) Glycogenolysis in the liver. The liver glycogen supplies the blood with glucose during the first 18-20 hours of fasting.

(B) Gluconeogenesis. It starts effectively after 4 hours of fasting and becomes the main source of blood glucose after the liver glycogen has been depleted (after 18 hours of fasting).

Control of blood glucose level :

The blood glucose level in a normal person is kept within the normal range. This blood glucose homeostasis is controlled by the following factors :

(A) Hormones

(B) The liver.

(C) Extrahepatic tissues as the gastrointestinal tract and the kidney.

(A) Hormonal control of the blood glucose level.

1. Insulin hormone.

Insulin is a protein hormone. It is secreated by the ß-cells of islets of langerhans of the pancrease.

Insulin is the only hormone that lowers the blood glucose level "hypoglycaemic hormone" through the following actions.

1. Insulin stimulates glucose uptake by the muscle and adipose tissue cells.
2. Insulin stimulates glucose utilization for oxidation, glycogenesis and lipogenesis .
3. It inhibits production of glucose by the process of glycogenolysis and gluconeogenesis in the liver.

4. It stimulates protein synthesis , thus decreasing the amino acids available for gluconeogenesis.

   Insulin secretion increases after meal

2. Glucagon.

It is a polypeptide formed of 29 amino acids .

It is secreted from the α-cells of islets of langerhans of the pancrease.

It increases the blood glucose level by inhibiting the glucose uptake and utilization by the tissues and by stimulation of glycogenolysis and gluconeogenesis in the liver.

Glucagon secretion increases during fasting.

3. Adrenaline

* It is derived from the amino acid Tyrosine.
* It is secreted from the suprarenal medulla.
* It increases the blood glucose level by inhibiting the glucose uptake and utlization by the tissues and also by stimulation of glycogenolysis in muscles and gluconeogenesis in the liver. Adrenaline inhibits the ß-cells of the pancrease that secrete insulin.
* Adrenaline secretion increases in stress conditions leading to stress hyperglycaemia.

4. Thyroxine "T4".

*Thyroxine is derived from Tyrosine amino acid.

* It is secreted by the thyroid gland.
* It increases the blood glucose level by increasing .

  The absorption of glucose from the intestine. It also stimulates glycogenolysis and gluconeogenesis and inhibits glycogenesis and lipogenesis.

*Thyroxine stimulates the catabolism of insulin by stimulation of the insulinase enzyme in the liver .

5. Glucocorticoids " as cortisone".

* Glucocorticoids are steroid hormones derived from cholesterol. They are secreted from the suprarenal cortex.
* They increase the blood glucose level by stimulating gluconeogenesis in the liver. They also inhibit the glucose uptake and oxidation and lipogenesis .
* Medication with corticosteroids can lead to hyperglycaemia and even glucosuria, and should therefore be taken with great caution in diabetic patients.

6- Growth hormone " diabetogenic hormone"

* It is a protein hormone secreted by the anterior pituitary gland.
* Growth hormone increases the blood glucose level by stimulating gluconeogenesis and by inhibiting the glucose utilization by the tissues. Prolonged administration of growth hormone as during treatment of dwarfism or chronic increase of growth hormone secretion as in gigantism or acromegaly may result in diabetes mellitus due to exhaustion of the ß-cells.

The control of the blood glucose level depends on the balance between the action of insulin and the anti-insulin hormones. After carbohydrate meal, the level of insulin increases to enhance glucose utilization while on fasting, the levels of the anti-insulin hormones " glucagon, adrenaline, glucocorticoids, thyroxine and growth hormones " are increased in the blood to elevate the blood glucose level to normal.

B. Role of the liver in Regulation of bood glucose level

The liver is called glucostat because it plays an important role in regulation of blood glucose level.

* In cases of hyperglycemia, the liver tries to decrease the elevated blood surgar by :

(a) Glycogenesis , (b) oxidation, (c) lipogenesis .

* In cases of hypoglycaemia, the liver increases the blood glucose level by :
(a) glycogenolysis , (b) gluconeogenesis

(C) Role of the kidney.

The kidney prevents the loss of glucose in the urine until the blood glucose level reaches the renal threshold which equals 180 mg/dl. Above this level the kidney allows the glucose to escape in the urine , a trial to reduce the elevated glucose level in the blood. Gluconeogenesis occurs in the kidney during fasting.

Diabetes Mellitus

Diabetes = increased urine volume.

Mellitus = the urine contains sugar " sweet urine "

Diabetes Mellitus is a metabolic disorder caused by deficiency of insulin hormone, whether absolute deficiency or relative deficiency due to increased production of the anti-insulin hormones. A decrease in the number of insulin receptors on the cells as in some cases of obesity leads to inefficiency of the insulin action and diabetes mellitus.

Diabetes mellitus causes metabolic disturbances in carbohydrate , lipid, protein water and electrolyte metabolism with disturbances in the acid-base balance.

[I] Effects on carbohydrate metabolism.

(a) Hyperglycaemia and glucosuria.

The deficiency of insulin hormone causes decrease in glucose uptake and utilization by the tissues, which results in increase in blood glucose level and detection of glucose in urine " glucosuria " if the level exceeds 180 mg/dl " renal threshold".

(b) Polyuria or increase in the urine volume is caused by the osmotic effect of the glucose that escape in the urine.

(c) polydepsia or increased drinking due to the dehydration caused by osmotic polyria.

(d) Polyphagia or excessive eating due to the increased hunger sensation . This is due to the reduced uptake of glucose by the satiety centre in the brain.

(e) Weakness. The uncontrolled diabetic patient is weak due to decreased ATP formation from glucose.

[2] Effects on lipid metabolism.

The diabetic patient depends more on lipids for energy production. The stored lipids in the adipose tissue are mobilised and lipolysis is increased. The released glycerol is changed to glucose by gluconeogenesis while the fatty acids are oxidized to give energy and acetyl COA. Part of this acetyl COA is used up in the kreb's cycle while the rest is changed into chalesterol leading to Hypercholesterolaemia and into ketone bodies leading to ketosis. In ketosis the ketone bodies " acetoacetic acid, ß-hydroxy buteric acid and acetone" are increased, leading to metabolic acidosis because the bicarbonate is consumed to buffer these ketoacids.

[3] Effects on protein metabolism:

The uptake of amino acids into the cells especially the muscle cells is decreased and the protein synthesis is inhibited. This effect causes wasting of the muscles and decreased immunity and delayed healing of wounds which depend on norml rate of protein synthesis.

[4] Effects on water, electrolyte and acid-base ballances.

The glucosuria leads to osmotic polyuria, dehydration with excessive drinking " polydepsia". The increased production of ketoacids causes acidosis "metabolic ketoacidosis". The Na^+, K^+ and ammonia are lost in urine in abnormally larger amounts.

Diagnosis of Diabetes Mellitus (D.M.):

[1] Symptoms : Polyuria , polydcpsia,polyphagia, weight loss and poor healing of wounds.

[2] Signs :

* Hyperglycaemia .

A. Fasting blood glucose level above 140 mg/dl or 2 hours post prandial level of 200 mg/dl or more or a random glucose level more than 200 mg /dl are criteria for diagnosis of D.M .

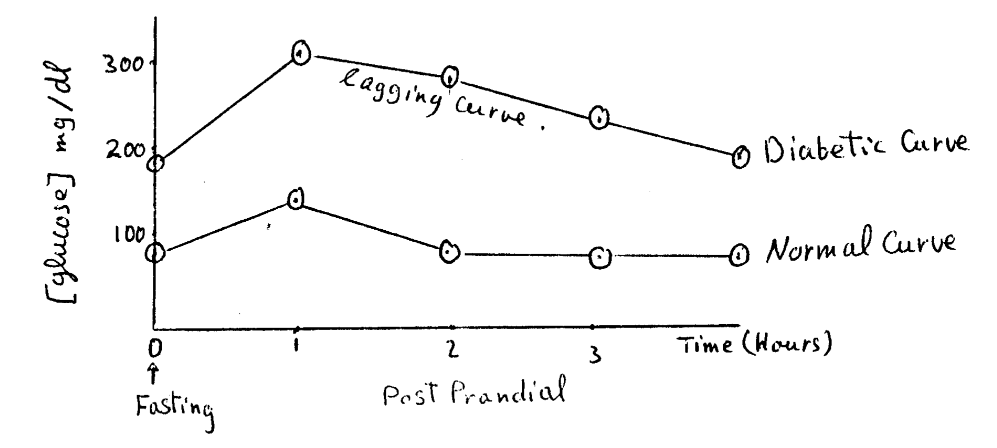
* Glucosuria.
* Ketonaemia and ketonuria.

Testing the tolerance or the utilization of glucose is the best method for diagnosis of D.M.

Glucose tolerance test :

*This test is used for diagnosis of D.M.

1. The patient should fast for 8-12 hours " overnight fast".
2. Blood sample and urine sample (fasting samples) are taken.
3. 50-75 g glucose are given orally to the patient (1 g/kg body wt.)
4. Blood and urine samples are taken every hour for 4 hours.
5. Determine the glucose concentrations in blood and draw a curve to show the relation between glucose concentation in blood and the time.



Normal curve:

* Fasting level is 80 mg/dl.
* 1 hour post prandial 150 mg /dl.
* 2 hours post prandial (2 hPP) 80 mg/dl (as the N. fasting).
* 3 hours post prandial the level is constat at the fasting level.

Diabetic curve :

*Fasting level : higher than normal (> 150 mg/dl)
* 1 h pp : above 180 mg/dL glucose appears in urine .
* 2 h pp. : higher than the fasting level (lagging curve).

Ttypes of Diabetes Mellitus :

1. **Type I** : Insulin Dependent D.M. (I.D.D.M.)

* It appears in young age.
  (Juvenile D.M.)
* Total destruction of ß-cells by disease like viral disease or autoimmune.
* It depends on Insulin in the treatent due to the complete absence of Insulin secretion.

2. **Type II** : Insulin Independent D.M. (I.I.D.M.)

* It appears in adults.
  (above the age of 40).
* The ß-cells are present but the secretion of Insulin is decreased .
* The treatment is based on :
  (A) Diet (decrease carbohydrates and lipids)
  (B) Oral hypoglycaemic drugs (Anti Diabetic drugs) as sulphonyluria.
  (C) Insulin is used Only on complications.

Diabetic coma :

2 types of comas can occur in diabetic patients.

Hyperglycemia :

* It is caused by neglecting the treatment with over intake of food.
*Bloo glucose level is very high above the ormal (>300 mg/dl).
*The patient is dry due to dehydration.
* Hyperventilation and rapid weak pulse.
*Acetone smell in breath and urine
* Ketones appear in urine.

***Treatment**

by Insulin

Hypoglycaemic

*Caused by over treatment with insulin or antidiabetic drugs with neglecting food intake.
* Blood glucose level is very low (<50 mg/dl.)
*The patient is wet due to excesive sweating .
* No hyperventilation . The pulse is rapid and strong.
* No acetone smell .
* No ketones in urine.

Treatment

by glucose infusion.

Glucosuria :

* It is the presence of glucose in a detectable amounts in the urine .
* It is followed by polyuria. (Osmotic diuresis).
* Causes :

1. Diabetes Mellitus :

glucose level in the blood above 180 mg/dl(renal threshold) will cause glucosuria. D.M. is due to defeciency of Insulin (in amount or activity) that causes decrease in glucose utilization and hyperglycaemia.

2. Renal glucosuria (Innocent diabetes).

The patients has low renal threshold for glucose (< 180 mg/dl) It may be less than 120 mg/dl and in this case glucose appear in urine after eating or even in the fasting.

3. Hormonal causes as in :

a. Increased growth hormone. G.H. is diabetogenic as in- Acromegaly Giagntism and Therapy with G.H.

b. Increased glucocorticoids as cortisone

They cause hyperglycaemia and glucosuria. They stimulate gluconeogenesis. Increase in glucocorticoids occurs in cushing disease and Therapy with cortisone.

c. Stress glucosuria due to increase in adrenaline and nor-adrenaline.

Types of Diabetes :

1. Diabetes mellitus.
2. Innocent diabetes.
3. Hormonal diabetes. as in increased glucocorticoids (Cushing disease) Increased growth hormone, Increased adrenaline and Noradrenaline.
4. Diabetes Insipidus

It is an increase in the urine volume due to decrease in the Anti Diuretic Homone (ADH) = vasopressin of the posterior pituitary.

In diabetes insipidus the urine volume is greatly increased (may be more than 10 liters per day). The urine has low specific gravity .No sugar in urine.

Hypoglycaemia :

* Hypoglycaemia occurs when the blood glucose level becomes lower than fasting levels " below 70 mg/dl" . Symptoms of hypoglycaemia develops when the blood glucose level is below 50 mg/dl.

The manifestations of hypoglycaemia.

1. Sweating , sense of hunger, tachycardia and termors. All the symptoms are caused by increased adrenaline secretion.

2. Confusion , headache, coma and convulsions. These symptoms are caused by inability of the brain to get energy from glucose.

* The causes of hypoglycaemia are:

1. Hyperinsulinism.

an increase in the amount of insulin in the blood above the normal a 4-24 μU/ml." may be caused by one of the following:

(a) An over dosage of insulin with missing a meal.

(b) An over dosage of insulinogenic drugs as the sulphonylureas that stimulate the ß-cells.

(c) Inslinoma or tumor of the ß-cells that produce large amounts of insulin.

2. Addison's disease.(Adrenocortical insufficiency),the deficiency of glucocorticoids causes a decrease in gluconeogenesis during fasting and causes an increase in the glucose utilization.

3. Anterior pituitary hypofunction.

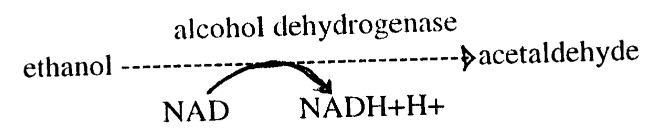
The level of growth hormone , TSH and ACTH are reduced . All these hormones elevate the blood glucose level .

4. Hypothyroidism.

The decrease in thyroxine level causes inhibition of glycogenolysis and gluconeogenesis and reduce the intestinal absorption of glucose.

5. Alcoholism:

Alcohol consumption causes the NAD to convert to NAD+H+ . This change in the NAD level causes inhibition of gluconeogenesis.



6. Hereditary fructose intolerance.

Fructose or sucrose ingestion induce hypoglycaemia. The cause is the deficiency of aldolase B enzyme that increases fructose-l-phosphate. The latter substance inhibits the glycogen phosphorylase, thus inhibiting glycogenolysis.

7 . Glycogen storage diseases as von Gierk's disease due to lack of glucose-6-phosphatase and inhibition of glycogenolysis.

8. Acute and severe liver diseases especially when the disease is extensive. The glycogen storage and gluconeogenesis occuring in the liver are decreased.

LIPID METABOLISM

Lipids in our food include:

1. Triacylglycerol (TG)
2. Cholesterol ester
3. Phospholipids

The fat soluble vitamins (A,D,E,K) are included in the fat components of the food.

These three lipid components need to be digested into simpler forms to be absorbed into the blood .

Digestion:

1. In the mouth.

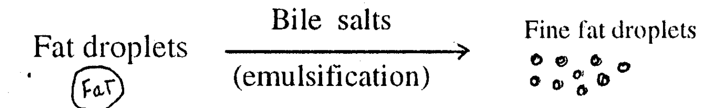
Some digestion occurs in infants by the lingual lipase. No digestion in adults.

2. In the stomach.

some digestion occurs in infant stomach by a gastric lipase (pH of infant stomach is 5) while the pH in adult stomach is unsuitable for the activity of this enzyme.

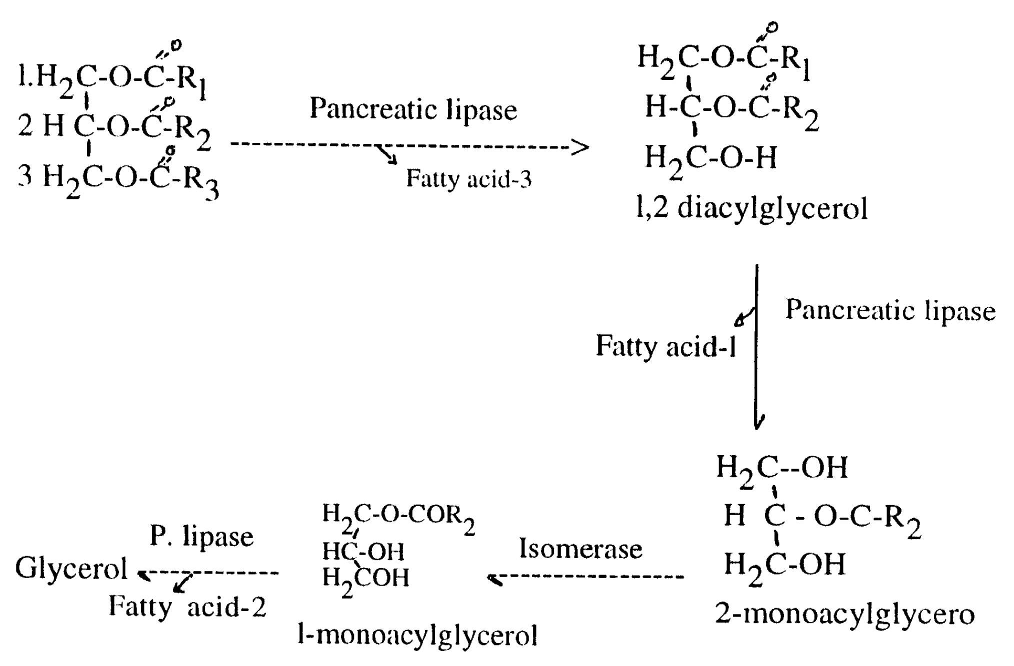
3. In the upper small intestine. A-digestion of TG .

* Bile salts (in the bile) causes emulsification of the fats. The big fat droplets change into very smaller ones, increasing the surface area for the activity of the enzyme . Bile salts also activates the lipases.



* The pancreatic lipase digests the triacylglycerol. This lipase is secreted by the pancrease and its secretion is stimulated by vagus stimulation and by the hormone pancreozymin of the duodenum.

The pancreatic lipase acts on the triacylglycerols to break down the ester bond in position 1 and 3 to produce 2-monoacylglycerol. The fatty acid in position 2 is then transferred to position 1 by isomerase to be acted upon by the lipase.



*The pancreatic lipase is secreted as inactive lipase that is activated by 1-bile salts, 2-colipase protein produced by the pancrease and by Ca^{++}.

*78% of the lipids are partially hydrolysed into 2-monoglyceride mainly and less amounts of diglycerides .

*22% of the lipids are hydrolysed completely into glycerol and 3 fatty acids.

(B) Digestion of phospholipids by phospholipases

Phospholipids --Phospholipases (Pancreatic & Intestinal)--> alcohol (glycerol) + Fatty acids + Phosphoric acids + Choline or other N.bases

C. Digestion of cholesterol-esters

Cholesterol esters --------------> cholesterol+ fatty acid.
(Cholesterol esterase, Pancreatic)

LIPID ABSORPTION

The products of lipid digestion includes

(A) Water insoluble components as

* Long chain fatty acid
* Diglyceride
* Monoglycerides
* Cholesterol

(B) Water soluble materials

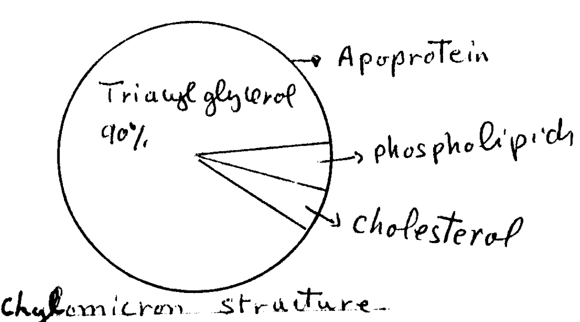
* Phosphoric acid.
* Choline, serine, ethanolamine
* Short-chain fatty acids.
* Glycerol.

Water insoluble products are mixed with some bile salts and lecithin to form micelles that can be carried in the water medium inside the intestinal lumen to reach the cells of the mucosa. They are absorbed passively through the lipid part of the cell membrane.

Also the water soluble products can pass easily through the cell membrane.

Inside the cytoplasm of the mucosa cells, TG, cholesterolester and phospholipids are resynthesized. They are surrounded by protein layer Apoprotein (AP) which transport them through the watery medium in the cell to reach the serosal surface of the mucosa cells this structure is called chylomicron .Chylomicrons (lipoprotein) are absorbed through

the lymphatics " lacteals" to the thoracic duct and then to the systemic blood.



chylomicron structure

-Chylomicrons in the blood causes milkness , turbidity of the plasma due to the high content of TG (85%-90%).

-Lipoprotein lipase is an enzyme produced by the endothelium of blood vessels . It acts within the blood vessles on TG of cylomicrons.

TG of chylomicron ----------------------> (Lipoproteins lipase) Fatty acids + glycerol

The chylomicrons turn to chylomicron remnants to be taken up by the liver cells.

*This enzyme is also called plasma clearing factor.Lipoprotein lipase is stimulated by Heparin, APO- C-II, Insulin, glucose and inhibited by Fasting, adrenaline "stress" and ACTH.

Plasma Lipids :

Lipids in the plasma are carried as lipoproteins.

Level :

Total plasma lipids
400-700 mg/dl

*Cholesterol 150-200 mg/dl.
*Triglycerids < 165 mg/dl
*Phospholipids 150-200 mg/dl.
*Free fatty acids 10-30 mg/dl.

Fate of absorbed lipids:

1. Oxidation for energy production
2. Building up the tissue (membranes) (Tissue lipids).
3. Storage in adipose tissue (depot fat)
4. Synthesis of lipids in adipose tissue, in mammary gland. and the liver.
5. Secretion by the sebaceous glands as sebum. TG is secreted by the lactating memmary gland.

Steatorrhoea = fatty diarrhoea

* It is a disease characterised by maldigestion and malabsorption of fats. The undigested fats remain in the stool.

* The fat content of the stool increases above normal (> 6 g/24 hour).

***Causes** :

1. Defect in secretion of bile salts as in obstructive liver disases. Bile salts play important role in :
   (a) Digestion of fats by emulsification
   (b) Absorption of fats by micellar formation.
2. Defect in the secretion of the pancreatic lipase as in acute and chronic pancreatic diseases.
3. Defect in the absorptive intestinal cells as in caeliac disease and non-tropical sprue these two diseases are characterised by diarrhoea due to destruction of the intestinal epithelium. The disease is caused by allergy against the protein of the wheat "glutelin".

***Effects :**

1. Deficiency of essential fatty acids as (linoleic, linolenic, arachidonic and clupanodonic).
2. Deficiency of fat soluble vitamins (Vitamin A,D,E,K)

3. Decrease in calcium and iron absorption due to formation of insoluble soaps with fatty acids.

Treatment :

-Treatment of the cause.

-Tablets containing lipases.

Lipoproteins

* The lipids in the plasma are carried by proteins (Apo-proteins) to form the different lipoproteins.

* The lipoproteins include :

1. Chylomicrons.
2. Very Low Density Lipoprotein = VLDL = pre ß-lipoprotein.
3. Low Density Lipoprotein = LDL = ß-lipoprotein.
4. High Density Lipoprotein = HDL = α -lipoprotein
5. Non Esterified Fatty Acid (NEFA) with albumin.

Chylomicrons:

* They are formed in the cells of the intestinal mucosa during absorption of digested fats.

* They contain Apo-protein ß-48 in (nascent chylomicron). It accepts other apoprotein in the circulation as Apo-C-II from HDL to become mature. Apo-E occurs in chylomicron remnants.

* It contains Triacylglycerol (TG), 85% -90% of the total lipids and small amounts of cholesterol ester and phospholipids.

-Chylomicrons transport the ingested lipids from the intestine to the blood.

- The triglycerides present in chylomicrons are hydrolysed by lipoprotein lipase within the blood vessels.

Lipoprotein lipase is stimulated by:

- Heparin - Glucose - Insulin - Apo C-II

It is inhibited by :

- Fasting.

- Adrenaline
- Stress

The chylomicrons are changed under the effect of lipoprotein lipase into chylomicron remnants .

Chylomicron remnants are taken up by liver cells. Apo-E in chylomicron is attached to specific receptors on the membrane of the liver cells . The liver cell lyses the remnants and utilizes its contents. Fatty acids released by lipoprotein lipase are carried by albumin in the plasma.

2.Very-Low Density Lipoprotein = VLDL=Pre ß

* VLDL are formed in the liver cells.
* It contains Apo-protein - B-100 . It also accepts other apoproteins as Apo-C-II and E in the circulation from HDL.
* It contains mainly triacylglycerols (56%) but less than that present in chylomicron. It contains more amounts of phospholipids (20%) and cholesterol ester (23%).
*It ransports lipids (mainly TG) from the liver cells to the peripheral tissues. Inhibition of its synthesis causes fatty accumulation (fatty liver).
* In the circulation, lipoprotein lipase hydrolyses the triglycerides of the VLDL into fatty acids and glycerol. The VLDL changes , then into Intrermediate Density Lipoprotein (IDL) which undergoes changes in its composition to form LDL.

```
       Lipoprotein lipase
VLDL ----------------------> IDL ----------> LDL
        ↘           ↘
        FA     glycerol
```

3. **Low Density Lipoprotein** = LDL = ß-lipoprotein .

*It is formed mainly in the peripheral circulation from VLDL catabolism through IDL formation.

Lipoprotein lipase
VLDL ---------------------> IDL -----------------> LDL
FA glycerol

It is also formed in the liver . it contains apoprotein B-100

*It contains 60% of the cholesterol content of the plasma.

It also contains variable amounts of phospholipids and triacylglycerols.

* It tansports the cholesterol from the liver to the tissues to be metabolised or stored as cholesterol ester.

* LDL-receptors are glycoproteins present on the surface of the peripheral tissue cells and also on the liver cells. They are specific to apo-B-100 and Apo E.

The LDL is attached toLDL-receptors to form LDL-receptor complex on the surface ofcell membrane. The LDL-receptor complex is internalized into the inside of the cell and then lysed by lysosomal enzyme to set free the cholesterol.

Cholesterol inside the cell undergoes the following :

1. It can be used up for the formation of biological membrane.
2. It can be used for the synthesis of (a) bile acids in liver cells
   (b) steroid hormones in endocrine cells.
   (c) Vit. D3 in the skin .
3. It can be stored as cholesterol ester.

Cholesterol+ Acyl COA ---------ACAT---------> cholesterol ester + COASH

N.B : ACAT = Acyl CoA- Cholesterol Acyl Transferase.

When the amount of cholesterol increases inside the cell, the LDL-receptors decrease in number . This phenomenon is called down regulation. In this Case the cholesterol increases in the blood due to decrease in its utilization by tissues. The rise of cholesterol level in the cell also inhibits the enzyme HMG-COA reductase necessary for the synthesis of cholesterol.

LDL in diseases :

* The desirable level of LDL -cholesterol should be less than 130 mg/dl
* High levels of LDL-cholesterol predisposes to atherosclerosis high levels of LDL occurs in :
  1. Uncontrolled Diabetes Mellitus.
  2. Smoking.
  3. Alcoholism.
  4. High level of saturated fatty acids in the food (as in solid fats)
  5. Lack of regular exercise.
  6. Genetic constitution-defect in LDL-receptors .
  7. High cholesterol diet.
  8. Hypothyroidism.

High Density Lipoprotein HDL = α-lipoproteins

Site of synthesis:

* It is formed in liver.

Composition:

* It contains Apoprotein-A " Apo-A" (Apo A -I , Apo-A-II, Apo A-IV) , Apo-C and E & D.

* It contains mainly phospholipids (40-45%) of its total lipids . Also it contain cholesterol ester and triacylglycerol.

Function:

* It transports cholesterol from the tissues to the liver to be excreted.

*It carries cholesterol as cholesterol ester.

* It contains the enzyme LCAT which adds fatty acid to the cholesterol to form cholesterol ester.

$$\text{Lecithin + cholesterol} \xrightarrow{\text{LCAT}} \text{cholesterol ester + lysolecithin)}$$

N.B.: LCAT = Lecithin - Cholesterol Acyl Transferase.

Apo A-II is stimulator to LCAT enzyme .

Fate :

* HDL is taken up by the liver cell to be hydrolysed and its contents " esp. cholesterol esters" are released and metabolised. The released cholesterol (50%)is changed mainly to Bile Acids.
  Bile acids are excreted in the bile as bile salts

Clinical importance :

*High levels of HDL-cholesterol (> 40 mg/dl) is protective againt atherosclerosis because HDL carries the cholesterol from the tissues to the liver for excretion .

Increase in the level of HDL can be achieved by:

1. Regular exercise.
2. Abstinence from smoking.
3. Abstinence from alcohol drinking.
4. High unsaturated fatty acids in the food and low saturated fatty acids .
   " i.e. oils instead of solid fat in food".
5. Nicotinic acid " Niacin " in high dose .
6. Genetic constitution.
7. Low cholesterol content in food.

Non-Esterified Fatty Acid = NEFA

* It is carried by albumin in the plasma (20 mg/dl) . The albumin fraction represent 99% of its structure .

* It is produced from lysis of Triacylglycerol in:

1. Adipose Tissue " lipolysis ".
2. VLDL by lipoprotein lipase.
3. Chylomicron " " "

*These free fatty acids are utilized by the different tissues either for energy production " ß-oxidation" or for reformation of T.G. or

esterification of cholesterol and in synthesis of phospholipids after being activated into acyl-COA.

Fatty acid + COASH --------Thiokinase--------> Acyl COA
ATP ↘ AMP + 2Pi

Lipids in the cell

Tissue fats	Depot fats
*They enter in the structure of the cell membranes *They include phospholipids, glycolipids and few cholesterol . The lipids are associated with proteins. * They are not affected by the feeding state. * They serve structural function . Its fatty acid arachidonic (20C) can be changed to the prostaglandins. Leukotriens and prostacyclines.	*They represent store of fat in the adipose tissue. * They are formed mainly of triacyl -glycerol (TG) and are rich in saturated fatty acids. *They increase in over -feeding state and decrease in the fasting state. *Their functions include (a) store of energy (b) Insulator under the skin. (c) Protection and support of internal organs.

LIPOLYSIS

*Triacyl glycerols (TG) in adipose tissue are continuously hydrolysed (lipolysis) and reformed (lipogenesis).

* The products of lipolysis are glycerol and fatty acids.

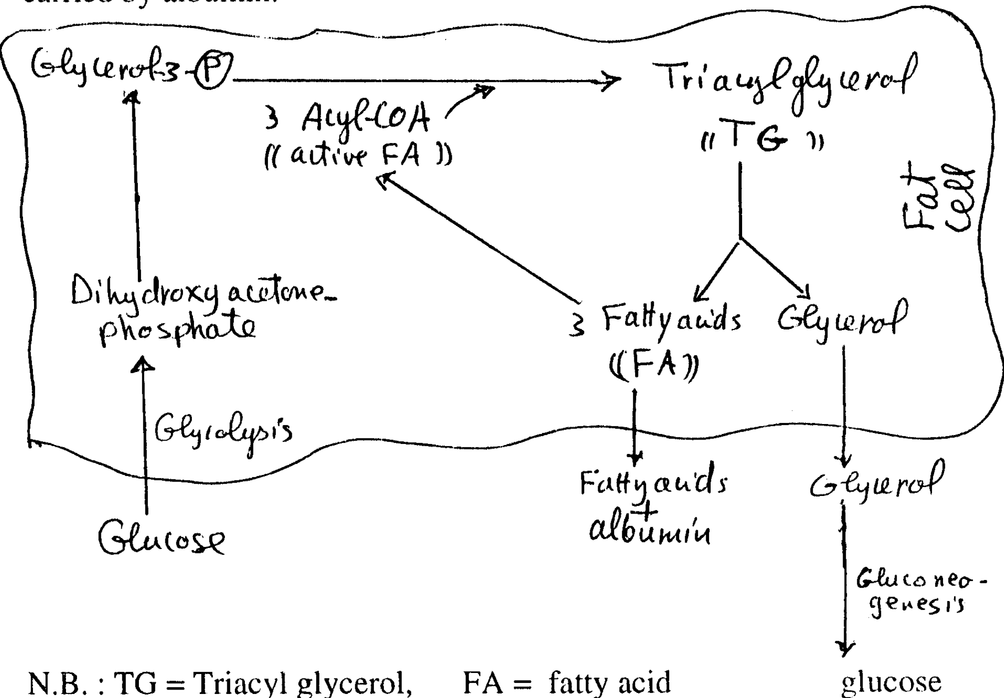
*Glycerol liberated inside the fat cells cannot be reutilized by the fat cells for formation of Triglycerides because the fat cells do not contain glycerol-kinase enzyme needed for activation of glycerol. This glycerol is released to the blood to be utilized in the liver and the kidney for reesterification to form (TG) or to change into:

Glucose -------> by gluconeogenesis

Pyruvate -------> by glycolysis.

*Free fatty acids (FFA) released by lipolysis are either used for resynthesis of TG after being activated into acyl COA.

(Fatty acid ------------------> acyl-COA) or released into the blood to be carried by albumin.

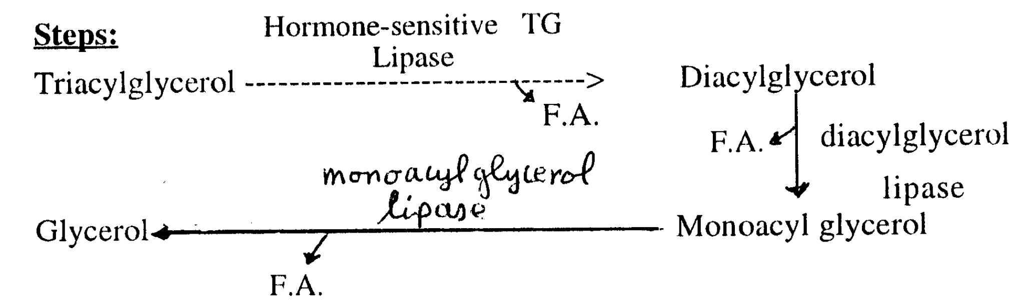


N.B. : TG = Triacyl glycerol, FA = fatty acid

Lipolysis is a continuous process. It increases in conditions of energy deficiency as in:

1. Prolonged starvation.
2. Low carbohydrate diet.
3. Diabetes Mellitus.

Steps:



Control of lipolysis :

Lipolysis is under control of the enzyme hormone-sensitive triacylgylcerol lipase (HS.Lipase).

This enzyme exist in two forms .

(a) Active which is phosphorylated

(b) Inactive which is dephosphorylated

Activation of (H.S. Lipase) .It is stimulated by :

1. Adrenaline and nor-adrenaline
2. Glucagon.
3. Growth hormones
4. ACTH, TSH, MSH
5. Methyl Xanthine " caffeine"
6. Fasting due to increase in antiinsulin hormones and deficiency of glucose.

Inhibition of (H.S. Lipase) occurs by:

1. Insulin
2. Glucose (carbohydrate feeding) .
3. Nicotinic acid and prostaglandin E_2

Lipogenesis

Definition:

Lipogenesis is the synthesis of triacyl glycerol (TG) mainly from glucose.

Site:

Lipogenosis occurs mainly in the liver, adipose tissue and lactating mammary glands.

Steps:

The synthesis of triacyl glycerol occurs in 3 Steps:

1- Synthesis of fatty acids from acetyl-COA which is mainly derived from glucose and less commonly from other sources. This step occurs in the cytoplasm and requires a complex enzyme system called fatty acid synthase complex and a source of hydrogen "$NADPH+H^{+}$".

2- Synthesis of active glycerol "glycerol-3-P" from glucose through the glycolysis.

3- Esterification of 3 fatty acid with glycerol to form triacylglycerol "TG". The triacylglycerol formed is secreted out of the liver as VLDL or stored as fat globules in the adipocytes "fat cells" in the adipose tissue.

Control: Lipogenesis is stimulated by excess carbohydrates in the diet and by insulin. Lipogenesis is inhibited by anti-insulin hormones and by low carbohydrates in food.

Oxidation of fatty acids

Oxidation of fatty acids produces large amounts of energy. it occurs in most tissues except the brain and RBC,s.

The Sources of fatty acids in the blood are:

1-Hydrolysis of triacylglycerol in the VLDL and chylomicron by the lipoprotein lipase .

2-Lipolysis of TG in adipose tissue.

Steps :

1-Activation of the fatty acid in the cytoplasm of the cells by conjugating the fatty acid with COA – SH. To form acyl – COA.

2-Transfer of the activated fatty acid from the cytoplasm to the inside of the mitochondria by the help of a substance called carnitiue and 3 enzymes. These enzymes are stimulated by fasting and inhibited by feeding.

3-B – Oxidation of the activated fatty acid "acyl – COA" in the mitochondria to produce acetyl- COA and reduced coenzymes "$FADH_2$ and $NADH+H^+$". The number of acetyl – COA molecules depends on the number of the carbons in the fatty acid. The reduced coenzymes "$FADH_2$ and $NADH+H^+$" are oxidized in the respiratory chain to produce ATP. The acetyle–COA produced is oxidized in the Kreb's cycle producing 12 ATP per cycle.

The β – oxidation of fatty acids is the main pathway for fatty acid oxidation. It is called β-oxidation because it involves splitting of acetyl-COA "2 carbons" from the fatty acid at the β - carbon.

Other minor pathways of fatty acid oxidation include ∝ - oxidation at the ∝ - carbon and omega "ω" oxidation at the omega carbon.

$$\text{"}\omega\text{"}\quad CH_3-CH_2 \text{-------------------} \overset{\beta}{CH_2}-\overset{\propto}{CH_2}-COOH \text{ (fatty acid)}$$

Ketone bodies Metabolism

The keton bodies "ketones" include :

1- Aceto acetic acid $\quad CH_3-\underset{\underset{O}{||}}{C}-CH_2COOH$

2- β -hydroxy buteric

3- Acetone

- Synthesis of ketone bodies "**Ketogenesis**" occurs in the mitochondria of liver cells from the precursor acetyl – COA. Ketone bodies are normally formed in small amounts "<1mg/dl". And the synthesis increases in all conditions that stimulate energy production from fatty acid oxidation as in starvation , low carbohydrate diet & untreated Diabetes Mellitus. The excess oxidation of fatty acids produce more acetyl-COA that change in part, to ketone bodies and cholesterol.
- The oxidation of the ketones to produce energy occurs in all extra hepatic tissues except the RBC,s and brain. The brain can utilizes ketones to get energy only in prolonged starvation. Oxidation of ketones is called **Ketolysis** .

• **Ketosis = Ketoacidosis**

Ketosis is a diseased condition that occurs when the production of ketones increases above the normal as in prolonged starvation, low carbohydrate intake and in untreated Diabetes Mellitus. In all these condition the level of ketones increase in the blood "ketonaemia" and is excreted in urine "ketonuria".

Ketosis causes Metabloic acidosis because the accumulated ketones are acids "as acetoacetic acid and β - hydroxy buteric "acid" which are buffered by the bicarbonates in the blood leading to decrease in the alkali reserve, hence the name metabolic ketoacidosis. Acetone is not utilized, but excreted in the expired air and in the urine. Acetone smell in the breath is used in diagnosis of diabetic ketoacidosis especially in comatosed patients.

Cholesterol Metabolism

Cholesterol is a type of lipids that is present only in animals. Cholesterol is a steroid compound.

Cholesterol "free"

Sources of cholesterol :

1– Exogenous sources "about 0.5 g / day" the foods that are rich in cholesterol include brain, egg yolk, liver and organ meats and butter.

N.B. Vegetable oils are free from cholesterol.

2- Endogenous Sources "about 0.5 g/day". Biosynthesis of cholesterol occurs in most tissues especially the liver and intestine. The source of all the carbons in cholesterol molecule is the active acetate "acetyl . COA" which is mainly produced from glucose or from fatty acids. An important enzyme "key enzyme" called HMG-COA reductase is the main enzyme in the pathway of cholesterol synthesis. This enzyme is stimulated by insulin and by carbohydrate feeding and inhibited by glucagon and fasting.

Mevastatin and lovastatin are drugs used to reduce cholesterol synthesis by inhibiting HMG-COA reductase enzyme.

Cholesterol level in blood

The normal total level of blood cholesterol is 150-200 mg/dl. 2/3 of cholesterol is esterified and 1/3 is free .

The blood cholesterol is mainly carried as LDL – cholesterol which represents 60% of the total cholesterol. The rest (40%) is present in HDL, VLDL, IDL and chylomicron.

Hypercholesterolaemia "blood cholesterol level higher than 200mg /dl" occurs in the following conditions:

1- Dietary causes.

Diet rich in cholesterol, carbohydrates and saturated fatty acids.

2- Uncontrolled Diabetes Mellitus.

The lipolysis increases in adipose tissue leading to release of more fatty acids that are oxidized to give more acetyl-COA which is used in part for synthesis of cholesterol.

3- **Obesity, coffee drinking and cigarette smoking** cause hypercholesterolaemia by a mechanism similar to that of DM.

4- **Hypothyroidism** . the decrease of thyroxine decreases the excretion of cholesterol .

5- Obstructive jaundice and nephrotic syndrome.

6- Inherited disorder of plasma lipoprotein

Type II and III according to Fredrickson classification .

Hypocholesterolaemia "blood cholesterol level less than 150 mg/dl" occurs in Hyperthyroidism and in liver diseases.

Excretion of cholesterol

Cholesterol is excreted as follows:

1-50% of cholesterol is oxidized in liver to bile acids "cholic and chenodeoxycholic" which are excreted from the liver as bile salts in the bile.

2-Part of cholesterol is excreted as free cholesterol in the bile. This part, when increased, is deposited in the gall bladder to form gall stone.

Atherosclerosis

Atherosclerosis is caused by deposition of cholesterol esters and other lipids especially triglycerides in the connective tissue of the arterial wall "intima". The macrophages phagocytose these lipid deposits and form foam cells. A process of inflammation in the intima ends with fibrosis in the wall of the arteries. The fibrosis "sclerosis" in the small sized arteries causes stenosis and predisposes to intravascular clotting. The atherosclerosis in cerebral and coronary arteries predisposes to cerebral and myocardial infarctions.

Atherosclerosis is often associated "correlated" with abnormalities in plasma lipids such as:

1- Increased serum cholesterol level "↑ LDL"

2- Increased serum triglycerides level "↑ VLDL or chylomicrons"

3-Low level of HDL. The ratio between LDL and HDL – cholesterol "LDL / HDL ratio" is more than 4.

Factors that predisposes to atherosclerosis includes :

1- Diseases associated with abnormalities in plasma lipids as Diabetes Mellitus, hypothyroidism, nephrosis, inherited hyper lipoproteinaemia .

2- Diet rich in cholesterol, saturated fatty acids and carbohydrates.

3- Cigarette smoking, excessive coffee drinking and emotional stress "high adrenaline". All these factors increase lipolysis and hence the synthesis of VLDL and LDL.

4- Lack of regular exercise.

5- Hypertension predisposes more to the complications of atherosclerosis .

Fatty Liver

- Fatty liver is a disease characterized by accumulation of abnormally large amounts of lipids "mainly triacylglycerol" inside the liver cells. In chronic conditions the distended liver cells may rupture and change to fibrous tissue causing liver cirrhosis.
- Causes of fatty liver : 2 main causes are :

1- Increased synthesis of triacylglycerol in the liver as in the following conditions:

(a)Overfeeding with fats and over feeding with carbohydrates.

(b) Increased lipolysis in the adipose tissue with mobilization of fats to direct synthesis of TG in liver cells. Lipolysis is increased in starvation, Diabetes Mellitus and low carbohydrate diet, Hyperfunction of pituitary gland and supra renalcortex.

(c) Alcohlism and pantothenic acid deficiency.

2- Decreased synthesis of VLDL in the liver. VLDL transports lipids formed in the liver cells "mainly TG" to the peripheral tissues. The main causes of decreased VLDL synthesis include deficiency of protein intake, liver toxins as chloroform & carbon tetrachloride, vitamin deficiency as choline & inosital and in some genetic defects as Abeta-lipoproteinaemia.

Treatment of fatty liver

Fatty liver is a curable condition. The aim of the treatment is to reduce the synthesis of Triglycerides in the liver cells and to help

the liver to release its content of triglycerides into the blood to be utilized. This is achieved through the following measures:

1- Avoid all the factors that increase the synthesis of triglycerides in the liver as high carbohydrate and fats in the diet, treatment of the diseases as diabetes mellitus that causes increased TG synthesis. Stopping alcohol drinking.

2- Administration of the agents that promote the synthesis of VLDL as choline, inositol, methionine, vitamin-B6 , Pantothenic acid and vitamin B_{12}. These agents are called lipotropic factors.

PROTEIN METABOLISM

The proteins in our food are either of animal origin as proteins of meat, egg and milk proteins or of plant origin as zein (maize) and gluten of the wheat. Proteins of animal origin have a high nutritional value because they contain all the essential amino acids in good amounts, while plant proteins are of low nutritional values because they lack one or more of the essential amino acids. The daily requirements of proteins:

Normal adults need 0.8-1 gram protein/kg body weight per day. At least half this amount should be of animal origin.

Digestion:

(A) In the mouth :

There is no digestion of proteins in the mouth .

(B) In the stomach :

HCl, Rennin and pepsin are produced in the stomach and play important role in the digestion of proteins.

1. HCl:

The HCl of the stomach causes dnaturation of the ingested proteins, the denatured proteins is easily digested by the proteolytic enzymes. HCl also starts the activation of the inactive pepsiogen into pepsin.

2. Pepsinogen

The pepsinogen is secreted by the chief cells of the gastric mucosa .

It is activated as follows : Pepsinogen $\xrightarrow{HCl}$ pepsin

Pepsinogen $\xrightarrow[\text{autoactivation}]{}$ pepsin

pepsin is an endopeptidase. It breaks down the peptide bonds in the middle of the protein chain especially at the aromatic amino acids.

3. Rennin . (pH optimum 4).

Rennin is secreted by the parietal cells of infants stomach. It acts on the soluble caseinogen of the milk to change it into insoluble calcium caseinate " cheese".

Caseinogen -------Rennin-----> Calcium caseinate "cheese"
Ca^{++}

This process help to delay the evacution of milk from the stomach of babies until its protein is properly digested by pepsin.

(c) In the upper small intestine:

Digestive juices secreted from the pancrease and the wall of the intestine contains the following proteolytic enzymes.

1. Trypsin "pancreatic"

It is secreted as inactive zymogen called trypsinogen. Its activation starts by the enzyme enterokinase of the duodenum and is completed by the active trypsin.

2. Chemotrypsin " pancreatic "

It is secreted as inactive chemotrypsinoen that is activated by trypsin into the active chemotrypsin. Both trypsin and chemotrypsin are endopeptidases. Trypsin is specific to the peptide bonds between the basic amino acids " as lysine and arginine" and the chemotrypsin is specific to the aromatic amino acid.

3. Elastase "pancreatic "

It is secreted as proelastase that is activated by Trypsin as elastase.

proelastase ---Trypsin-------> elastase

4. Carboxy peptidase " pancreatic "

It is secreted as procarboxypeptidase which is activated by trypsin.

Procarboxypeptidase -Trypsin-------> carboxypeptidase . It is an exopeptidase which acts on the peptide bonds at the carboxylic end of the polypeptide chain liberating free amino acids.

5. Aminopeptidase " intestinal"

It acts on the peptide bonds at the amino end of the polypeptide chain. It liberates free amino acids.

6. Tripeptidases and dipeptidases. " Intestinal" they act on the tripeptides and dipeptides forming free amino acids . They are present at the brush border of the intestinal cells.

The sequence of protein digestion by the proteolytic enzymes proceeds as follows.

Protein -----> proteose----> peptone ------> polypeptide

amino acid <----------- tri and dipeptides.

Absorption of digested prtoeins:

*Normally the amino acids are the absorbable form of the digested prtoeins. Intact proteins or polyptpides cannot be absorbed through the intestine except in infants who can absorb the IgG "immunoglobulin "G" present in the early milk or the colostrum and in cases of food allergy. In food allergy the intestine allows absorption of small amounts of intact proteins which act as antigen exciting an allergic reaction.

The absorption of amino acids occurs by a sodium dependent, carrier mediated active transport. There are specific carriers for the different groups of the amino acids . Neutral and aromatic amino acids share the same carrier.

Hart-Nup disease is an amino-aciduria caused by hereditary deficiency of the carrier protein for the neutral and aromatic amino acids in both the kidney and the intestine. In this disease severe deficiency of tryptophan causes pellagra.

Plasma amino acids :

* Level : 4-8 mg/dl.

* Sources :

1. Digestion of dietary proteins, the amino acids are absorbed to the portal blood to reach the liver then to the systemic circulation.

2. Endogenous protein turnover. Proteins in the cell undergo certain rate of Turnover. They are broken down and resynthesized .

* The level of plasma amino acids shows diurnal variation.

The highest level is at mid-day---> 8 mg/dl.

The lowest level is at mid-night ---> 4mg/dl.

Factors that effect the level of plasma amino acids include.

1. Hormones:

* Growth H, Insulin, Thyroid hormones stimulate the protein synthesis, thus decreasing the level of plsma amino acids .

* Glucocorticoids stimtulate protein catabolism, thus increasing plasma amino acid level.

2. Food components :

* Food rich in proteins increases their level. Defeciency of protein in food decreases their level.

* Carbohydrates have sparing effect for amino acids.

Utilization of amino acids :

(1) 85% of plasma amino acids are utilized for protein synthesis.

(2) Some amino acids are used to form important non-protein biological compounds, e.g.

- Tyrosine is used for synthesis of:

T4,T3 " Thyroid hormones"

adrenaline and noradrenaline.

dopamine.

Melanin pigment .

-Tryptophan is used for synthesis of:

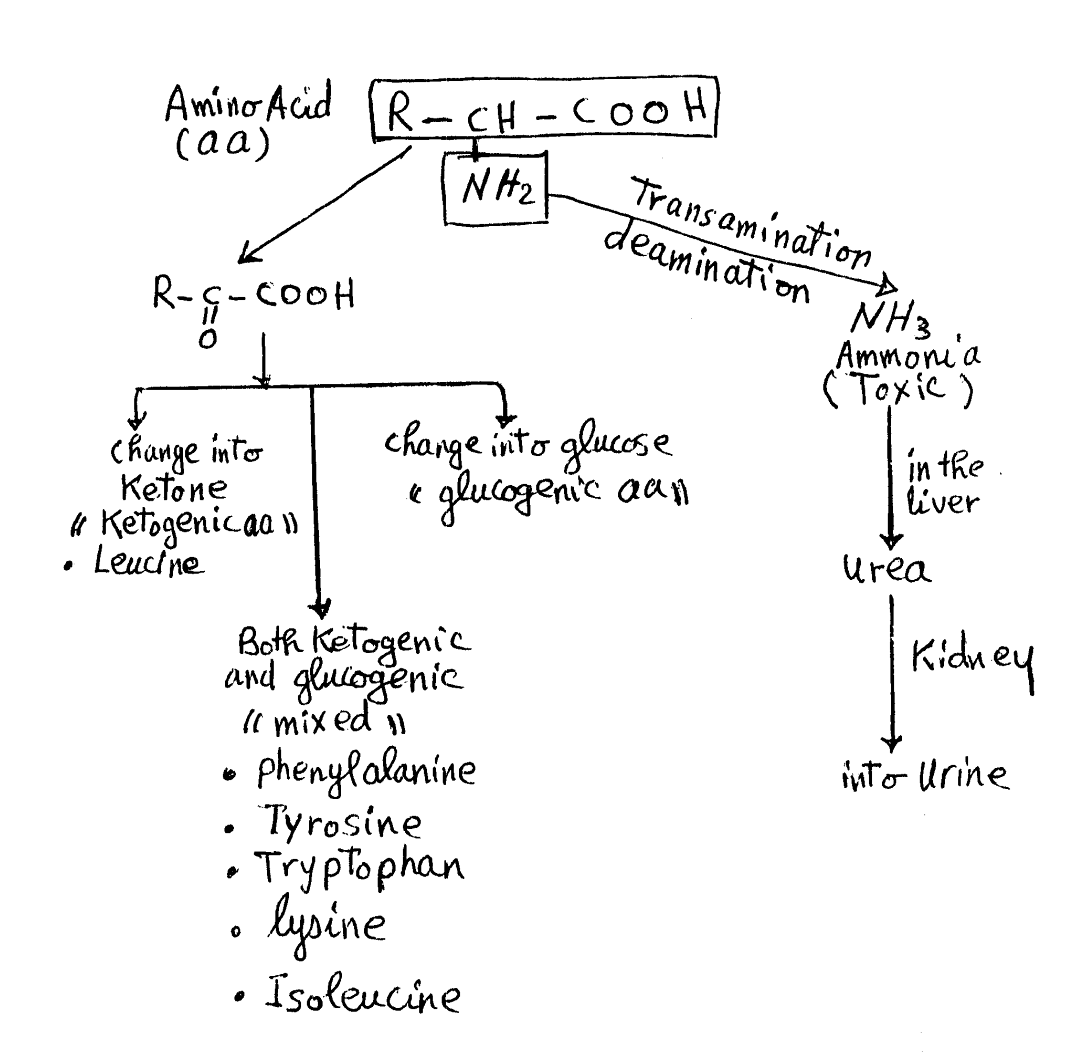
* Serotonin

* Melatonin

* Niacin

- Histidine decarboxylation give histamine

(3) Some amino acids are utilized for energy production.
15% of the total caloric requirement per day should be obtained from amino acids after removal of their amino groups as ammonia.



Transamination:

- It means transfer of amino group ($-NH_2$) from an amino acid to α-keto acid to form new amino acid and a new α-keto acid.
- The enzyme needed is a transaminase or "Aminotransferase" which require pyridoxal phosphate as coenzyme (PLP).
- Examples :

(1) GOT = glutamic Oxalacetic Transaminase

= Asparatate Transaminase (AST)

$$\begin{array}{l} COOH \\ | \\ CH\,NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} + \begin{array}{l} COOH \\ | \\ C=0 \\ | \\ CH_2 \\ | \\ COOH \end{array} \underset{PLP}{\overset{GOT}{\rightleftharpoons}} \begin{array}{l} COOH \\ | \\ C=0 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} + \begin{array}{l} COOH \\ | \\ CH-NH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

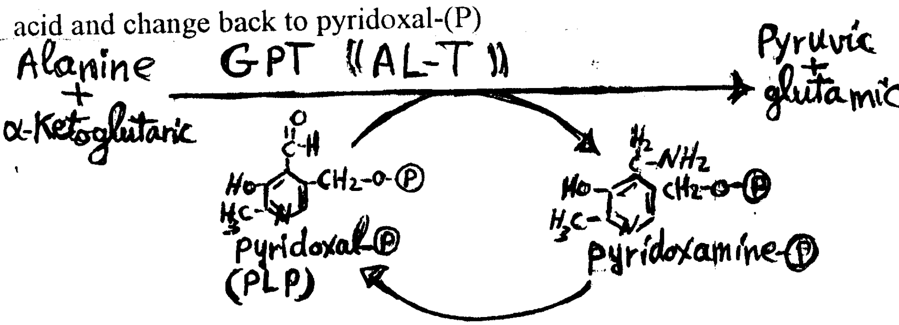
Glutamic oxalacetic α-keto glutaric Asparatic acid

(2) GPT = Glutamic Pyruvic Transaminase

= alanine Transaminase

$$\text{glutamic} + \underset{\text{pyruvic}}{CH_3-\overset{\overset{O}{\|}}{C}-COOH} \underset{PLP}{\overset{GPT}{\rightleftharpoons}} \text{α-keto glutaric} + \underset{\text{alanine}}{CH_3-\underset{NH_2}{\underset{|}{CH}}-COOH}$$

- GOT and GPT are present in most tissue especially in the liver and the heart. They increase above the normal level in the blood during diseases of the heart (especially GOT) and liver (esp. GPT).
- The coenzyme is pyridoxal phosphate. It is the carrier of amino group. It forms pyridoxamine-(P) that gives the amino group to the α-keto acid and change back to pyridoxal-(P)



Deamination

- Removal of amino group (NH_2) from amino acids as ammonia (NH_3).
- It occurs mainly in the liver and kidney.
- Types: deamination include:

(A) Non-Oxidative deamination

This type occurs in hydroxy-amino acids as Serine, Homoserine, Threonine, cysteine and homocysteine, each has its specific enzyme.

CH2 – CH – COOH dehydratase H_2O
OH NH2 → $CH_3 - C(=O) - COOH + NH_3$
Serine H_2O Pyridoxal-(P)
Pyruvic ammonia

(B) hydrolytic deamination

This type occurs in the kidney and in the muscles.

- In kidney : glutamine —glutaminase (H_2O, NH_3)→ glutamic

This is important in acid-base balance in cases of metabolic acidosis. The excess hydrogen ions (H^+) is bound to the released ammonia to form ammonium (NH_4^+) ion that is excreted readily in urine as ammonium salt.

- In the muscles :

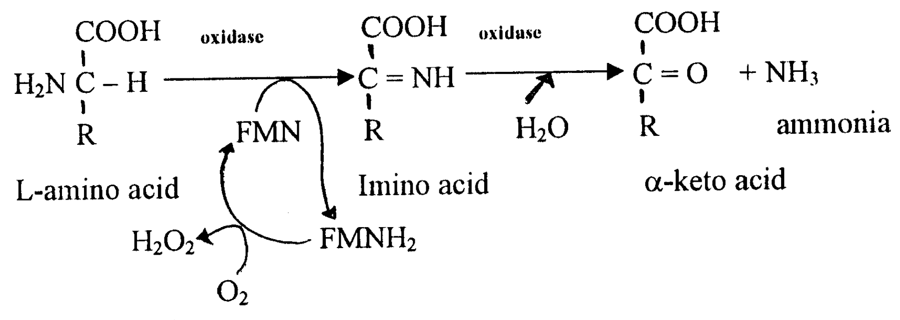
AMP acts as a source of ammonia in the muscles. It occurs during the catabolism of the purine nucleotude (AMP).

Adenosine monophosphate —deaminase (NH_3)→ Inosine monophosphate

(C) Oxidative deamination

[a] By amino acid oxidases (L & D)

The physiological importance of L and D amino acid oxidases are not fully known. Esp. D-amino acid oxidases because D-amino acids don't exist in large amounts in human cell. D-amino acids are present in bacteria and some antibiotics.



The L-amino acid oxidase uses FMN as coenzyme while D-amino acid oxidase uses FAD as coenzyme.

[b] L-Glutamaic acid dehydrogenase.

This enzyme is highly active and widely distributed in the tissues. It uses NAD or NADP as coenzyme.

H_2N–CH–COOH | CH_2 | CH_2 – COOH (L-glutamic) $\xrightarrow[H2O \quad NAD(P) \quad NAD(P)\,H+H+]{\text{glutamic dehydrogenase}}$ O = C – COOH / CH2 \ CH2 – COOH + NH_3

L-glutamic α-ketoglutaric + ammonia

Most amino acids transaminate their amino group to the a-keto glutaric to form glutamic acid which is then deaminated by this enzyme. The activity of this glutamic acid dehydrogenase is controlled by the levels of ATP, GTP and NAD(P). High level of ATP, GTP and $NAD(P)H^+H^+$ cause inhibition of this enzyme. High protein diet stimulates it, carbohydrates inhibits it.

Metabolism of Ammonia

Sources

1- Ammonia (NH_3) is produced by deamination of amino acids in the tissues.

2- Deamination of amino acids in the diet by the intestinal bacteria. This source increases after intestinal bleeding.

3- Hydrolytic deamination of AMP in the muscles.

Level of blood ammonia

Ammonia is normally present in a very small amount in the systemic blood. (10 – 80μg/dl). Ammonia is toxic to the brain and therefore it is rapidly removed from the blood by the liver mainly. The portal blood contains larger amounts of ammonia.

Fate of ammonia = (getting rid of ammonia)

Being toxic, ammonia is removed from the blood by the following means:

(1) Removal of ammonia produced in the kidney as ammonium salt in the urine.

Glutamine $\xrightarrow{\text{glutaminase}}$ glutamic + NH_3 $\xrightarrow{\pm H^+}$ $-NH_4$ in the urine.

(2) Formation of glutamic from α-ketoglutaric.

α-ketoglutaric + NH_3 $\xrightarrow[\text{NAD(P) H+H+} \quad \text{NAD(P)} \quad \text{H2O}]{\text{glutamic dehydrogenase}}$ glutamic

Other non essential amino acids can be fonned in a similar way.

(3) Formation of Glutamine from glutamic. This fate is very active in the brain and the kidney.

Glutamic + NH_3 $\xrightarrow[\text{ATP Mg++} \quad \text{ADP +Pi}]{\text{glutamine Synthetase}}$ Glutamine

This reaction is essential in the brain to get rid of the toxic ammonia. In the kidney tubules, the formation of glutamine is important in regulation of acid-base balance. The glutamine in the renal tubules is hydrolytically deaminated to produce ammonia which bind the excess H^+ and form ammonium ions in the urine, thus helping removal of H^+ in cases of metabolic acidosis.

(5) Ammonia is also used in synthesis of the purines and pyrimidines.

(6) Ammonia is changed into UREA in the liver. Urea formation is the main fate of the ammonia. Urea is the main end product of the amino acid catabolism. Urea is removed by the kidney in the urine and is the main non-protein nitrogenous compound (NBN).

Urea cycle:

Site : The liver. 5 enzymatic steps form the urea cycle, the first two steps occur in the mitochondria and the other steps occur in the cytoplasm of the liver cells.

Steps : the 5 steps are shown in the following figure.

Blood Urea Nitrogen (BUN):

The blood urea is determined as blood urea nitrogen and its normal level is 8 – 20mg/dl. This level increase in renal diseases as in renal failure. Uraemia is a sign of renal failure.

Control of Urea Formation

1 – The level of urea cycle enzymes are affected by the feeding state.

- High protein diet increases the level of the enzymes of the urea cycle.
- Protein free diet or law protein diet decreases the level of the enzymes of urea cycle.
- Starvation stimulates gluconeogenesis from amino acids. In this case deamination increase and the urea cycle is stimulated.

2– N-acetyl glutamate is a positive allosteric effector "stimulator" of the carbamoyl phosphate synthetase enzyme. The synthesis of N-acetylglutamate is stimulated by amino acids.

Hyper ammonaemia

It is defined as an increase in blood ammonia above the normal level (10–80 μg/dl). The causes are either acquired or hereditary.

(A) Acquried hyper ammonaemia

- Liver cell failure causes accumulation of ammonia in the systemic blood. The liver cannot change the toxic ammonia to the non-toxic urea.
- Liver cirrhosis and porto-systemic anastomosis cause mixing of the portal blood that contain higher levels of ammonia with the systemic blood. Liver cirrohosis may cause portal hypertension and bleeding of oesophageal varieces. The bacterial enzymes in the intestine act on the proteins in the blood and food inside the intestinal lumen liberating large amounts of ammonia that reach the diseased liver which fail to change the ammonia to urea.

(B) Inherited hyper ammoneamia

Deficiency of any one of the five enzymes of the urea cycle causes accumulation of the ammonia in the blood and toxicity to the brain. Inherited deficiency of the mitochondrial enzymes in step I and II namely, Carbmoyl – (P) synthetase and ornithine transcarbamylase are the most common.

Manifestations of hyper ammonaemia

- Vomiting, irritability and ataxia.
- Coma "hepatic encephalopathy".

Mechanism of hepatic coma.

High level of blood ammonia causes decrease in the level of α-ketoglutarate in the brain. This is because the brain detoxifies the ammonia by adding it to α-ketoglutarate to form glutamate as follows:

α-ketoglutaric + NH_3 —glutamic dehydrogenase→ glutamic
NAD(P) H+H+ NAD(P) H2O

This reaction occurring in the brain causes severe reduction in the level of α-ketoglutarate and inhibition of citric acid cycle leading to decrease in energy production (low ATP) in the brain and coma. On the other hand the production of GABA "γ-aminobuteric acid" is increased in the brain

Glutamic —decarboxylase→ (GABA) γ β α $CH_2\,CH_2-CH_2-COOH$ | NH_2
HOOC–CH–CH2–CH2–COOH | NH_2 PLP CO_2

GABA is an inhibitory neurotransmittor which may contribute to the mechanism of coma.

Treatment

1- Reduction of proteins in the food. The daily protein allowances are divided into smaller portions.
2- Enema to evacute the intestine from its contents that contain proteins.
3- Intestinal antiseptics as neomycin kill the intestinal bacteria and reduce the ammonia production in the intestine.

Nitrogen balance:

Proteins are the main source of nitrogen to the bodies. The nitrogen content of proteins is 16% which means that every 100 grams of protein contain 16 grams of nitrogen.

Normally the intake of nitrogen in the form of protein is equivalent to the nitrogen losses. The losses of nitrogen occur after catabolism of the amino acids. The routes of excretion of nitrogen are the urine (in the form of urea 14g / 24 hours, creatinine, uric acid, ammonia 0.79 / 24 hours), the stool as the proteins in the secretions and cell debris, the sweat as small amounts of urea and in milk, menstrual blood and hairs.

There are 3 states of nitrogen balance:

(1) Balanced state.

The intake of nitrogen is equal to the losses. This state occurs in healthy adults receiving normal protein intake.

(2) Positive nitrogen balance

The nitrogen intake is more than the nitrogen losses. The difference is retained in the tissues to be used in building up tissues.

Positive nitrogen balance occurs in the following cases :

1 – Growing children. 2 – Pregnant females

3 – Lactating females 4 – during convalescence

(3) Negative nitrogen balance

The nitrogen intake is less than the losses.

Negative nitrogen balance occurs in the following cases:

(1)– Severe burns (2)– chronic wasting diseases as malignancy, tuberculosis and typhoid.

(3)– Diseases causing loss of proteins as in haemorrhagic diseases and albuminuria (4)– Increased protein catabolim as in diabetes mellitus and hyper thyroidism.

Normal protein requirements:

The normal requirements per day ranges from 0.8 gram to 1 gram protein/Kg. Body weight. The Different animal sources of proteins contain different percentages of pure proteins as in the following example:

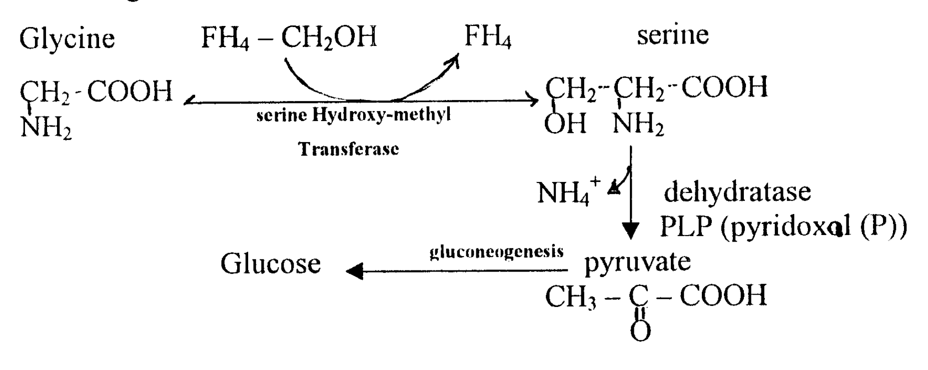
Protein Source	Protein content in 100g of the edible parts (gram).
Egg	11
Full cream milk	3.5
Full cream yoghurt	5
Full cream cheese	25
Double Creame cheese	15
Skimmed cheese	37
Tuna – Fish	24
Sardin – Fish	24
Gelatine	84
Ducks	15
Turky	23
Beef	22

Metabolism of Individual amino Acids:

Glycine

$$\underset{NH_2}{CH_2}\ COOH$$

- Non-essential amino acid
- Glucogenic



PLASMA PROTEINS

Plasma: It is the clear fluid that is obtained when blood is drawn into a tube containing an anticoagulant and is centrifuged. The blood cells sediment leaving the clear fluid (plasma) above.

Composition of the plasma:

The plasma is composed of water + all the dissolved substance in the whole blood.

The water is 90 – 92%

The dissolved substances include:

A- Organic compounds as :

1- Proteins (plasma proteins)

2- Lipids as lipoproteins

3- glucose and other sugars

4- Urea, hormones, ketone bodies, ...

B- Inorganic as: Na^+, Ca^{++}, Ng^{++}, HCO_3^- , Cl^-

Serum : It is similar to the plasma except that the blood has been allowed to clot.

i.e. It is defibrinated plasma (plasma without fibrinogen).

The differences between serum and plasma are :

Plasma	Serum
1-It contains the protein fibrinogen (0.2 – 0.4 g/dl)	1-It does not contain fibrinogen because it has been converted to fibrin clot (insoluble).
2-It is obtained by addition of anticogulant while collecting the blood	2-It is prepared by allowing the blood to clot.
3-It may be used as blood substitute (to replace blood).	3-It is not used as blood substitute.

Plasma proteins :

- Total level of plasma proteins is : 6.2 – 8.4 g/dl.
- Serum protein is about 0.2 – 0.4g/dl less than the plasma (due to fibrinogen)
- The measurement of total proteins is based on Biuret method.

Plasma proteins are separated into different fractions using Electrophoresis. These fractions are :

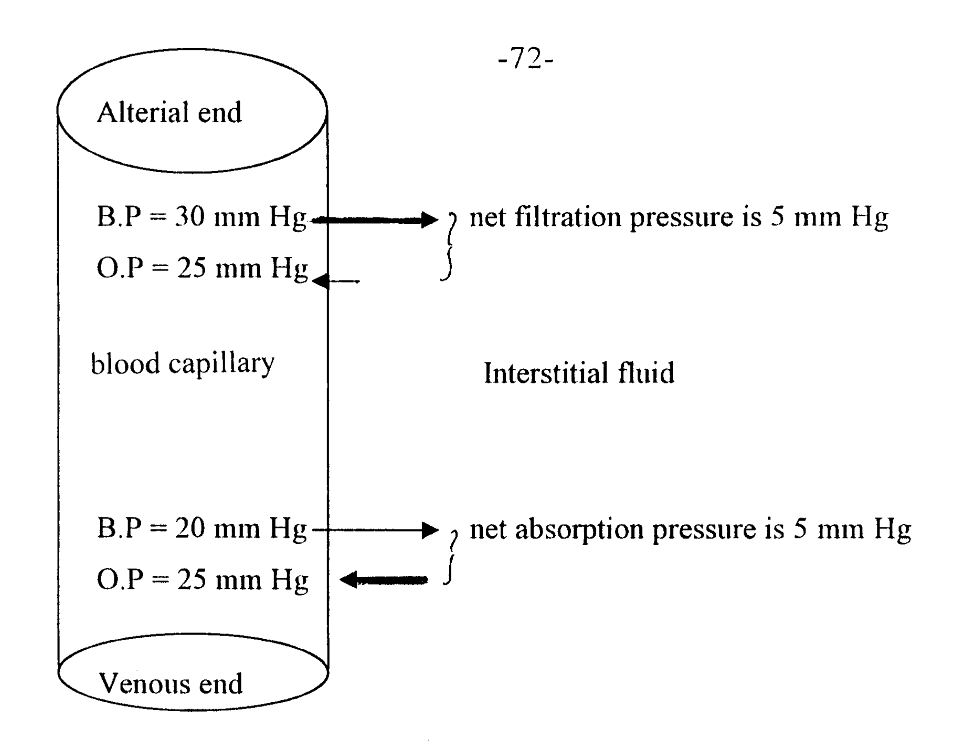
Type	Plasma level g/dl	% of total plasma protein	
1- Albumin	3.6 – 5.2	55	
2- α_1-Globulins	0.1 – 0.4	5	
			,prothrombin
α_2-Globulins	0.5 – 1.2	9	
β-Globulins	0.5 – 1.2	13	
Fibrinogen	0.2 – 0.4	7	
γ-Globulins	0.7 – 1.5	11 – 22	Antibodies

The total plasma globulin is 1.8 – 3.2 g/dl.

Functions of plasma proteins :

(1) They control the distribution of extracellular fluid between the blood and interstitial fluid by means of the osmotic pressure they produce.

The proteins of the plasma have large molecular weights. Therefore they cannot pass through the blood vessel wall into the interstitial fluid. They also draw water into the vascular bed and create osmotic pressure (oncotic pressure O.P.). Albumin provides 80% of the osmotic pressure effect of plasma protein due to its high concentration in the plasma and smaller molecular weight.



2 – They serve as carriers for various substances

- Albumin : It carries
- Most of the bilirubin
- Protein bound (non – ionised) calcium
- Hormones
- Many drugs as salicylates
- Many dyes introduced into the circulation as bromsulphthalein. Evans blue.
- Many metabolites as Free Fatty Acid (FFA).

- α_1 – Globulins : It carries
- carbohydrates (glycoproteins)
- High density lipoproteins HDL (α_1 – lipoprotein)

N.B. : α_1 – antitrypsin is the major α_1 – Globulin.

- α_2 – Globulins: It carries
- Haemoglobin that escapes into the plasma (Haptoglobulin)

- Copper (ceruloplasmin) it has oxidase activity.
- Carbohydrate (glyco proteins)
- Very low density hypoprotein (VLDL) (preβ)

N.B.: Prothrombin is an α_2 – Globulin.

-β – Globulins : It carries

- iron (Fe^{+++}) (Trans ferrin)
- low density lipoprotein (LDL) (β lipoprotein)
- Fat soluble vitamins
- Factor VIII

N.B. fibrinogen.

(3) Some proteins in the plasma are Hormones or enzymes (see Hormones)

(4) The γ- Globulins act as antibodies to protect the body against foreign proteins, Viruses, Bacteria.

<u>Gamma Globulins</u> (immuno – globulins)

γ – Globulin fraction contains the antibodies or immunoglobulins.

Total Level 0.7 – 1.5 g/dl in normal plasma

Site of formation : cells of the reticulo-endothelial system, lymphocytes and plasma cells.

Types: There are 5 classes of immunoglobulin listed in the following table.

Type	Percentage(%) of total immunoglobulins	Mole cular weightx10^{-3}
Ig G	80	150
Ig A	13	160, 320, 480
Ig M	6	900
Ig D	1	185
Ig E	0.002	200

Each class is made up of large number of individual ones.

N.B. Ig = Immunoglobulin

Hemoglobin Metabolism

It is a globular protein present in high concentration in the red blood cells. Being a chromoprotein, it confers its red color to the blood . Its concentration in the blood ranges between (12-18 g/dl) depending on age and sex. It is formed in immature erythrocytes in the bone marrow. It transports oxygen from the lungs to the tissues and carries CO_2 and protons to the lungs.

Structure :

Hemoglobin belongs to the class of conjugated proteins. Each molecule is formed of 4 heme units + globin (4 polypeptide chains) Heme is a ferrous protoporphyrin.

Heme group

M = methyl ($-CH_3$)

V = Vinyl ($-CH = CH_2$)

P = Propionic ($-CH_2 CH_2 COOH$)

Biosynthesis of hemoglobin

Synthesis of hemoglobin involves synthesis of the globin and synthesis of heme in the bone marrow and the liver cells.

Heme synthesis :

heme, the iron protoporphyrin of hemoglobin is synthesized as follows:

1. Condensation of succinyl COA with glycine in the mitochondria to form delta-amino levulinic acid "ALA" the reaction is catalysed by ALA-synthase which needs pyridoxal phosphate as conenzyme "PLP".

N.B.: Deficiency of vitamin B6 that forms pyridoxal phosphate is a cause of anaemia.

Glycine + succinylCOA --------ALA-synthase--------> δ-ALV
PLP, ↘ COASH, ↘ CO_2

2. In the cytoplasm, 2 ALA condense to form porphobilinogen.

2. ALA ------ALA - dehydrase------> porphobilinogen
Zn, ↘ $2H_2O$

3. 4 molecules of porphobilinogen condense to form uroporphyrinogen III mainly and few uroporphyrinogen I.

4 porphobilinogen ---Uroporphyrinogen III synthase---> uroporphyr inogen III

4. Decarboxylation of uroporphyrinogen III produces coproporphyrinogen III.

Uroporphyrinogen III ------decarboxylase------> coproporphyrinogen III
↘ $4CO_2$

The rest of the reactions for heme synthesis occur inside the mitochondria.

5. Oxidation of coproporphyrinogen III produces portoporphyrinogen III which is also oxidized to produce protoporphyrin III. Iron "Fe++" is then attached to protoporphyrin to form a heme molecule.

Coproporphyrinogen III --oxidase--> protoporphyrinogen III

Protoporphyrinogen III --oxidase--> protoporphyrin III

Protoporphyrin III + Fe^{++} --Heme synthase "Ferrochelatase"--> Heme

Control of Heme synthesis

The ALA-synthase enzyme is the rate limiting enzyme.

Inhibition of ALA-synthase is done by increase in heme level,while hypoxia stimulates the synthesis of heme.

Diseases retated to heme synthesis :

1. **Anaemia** is a decrease in hemoglobin concentration in the blood" Normal value is 16-18 g/dl "in males and 12-16 g/dl".in females. A major cause of anaemia is the decrease in the synthesis of hemoglobin due to :

(a) Deficiency of vitamin-B6 " pyridoxine". This deficiency inhibits the ALA-synthase enzyme.

(b) Lead toxicity. Lead inhibits the ALA-dehydratase enzyme which contain zinc.

(c) Iron deficiency. This is a very common cause of anaemia.iron deficiency is commonly caused by chronic blood loss. Iron deficiency affects the last step in heme synthesis that require the iron in the ferrous state.

(d) Deficiency of vitamin-B12 and deficiency of folate. Both vitamins are required for the synthsis of heme especially at the step for formation of the heme precursor succinyl-COA. A macrocytic anaemia is caused by deficiency of vitamin-B12 or folate.

(e) Severe deficiency of proteins in the food can cause an inhibition of the synthesis of the globin.

[2] Porphyrias:

Porphyrias are groups of hereditary diseases caused by deficiency of any of the enzymes needed for the synthesis of heme starting from step 3. The diagnosis of porphyrias is based on elevated ALA and porphobilinogen levels in blood and urine and assay of the deficient enzyme.

There are 3 types of porphyria according to the organ affected.

(a) erythropoetic porphyria.

(b) hepatic porphyria.

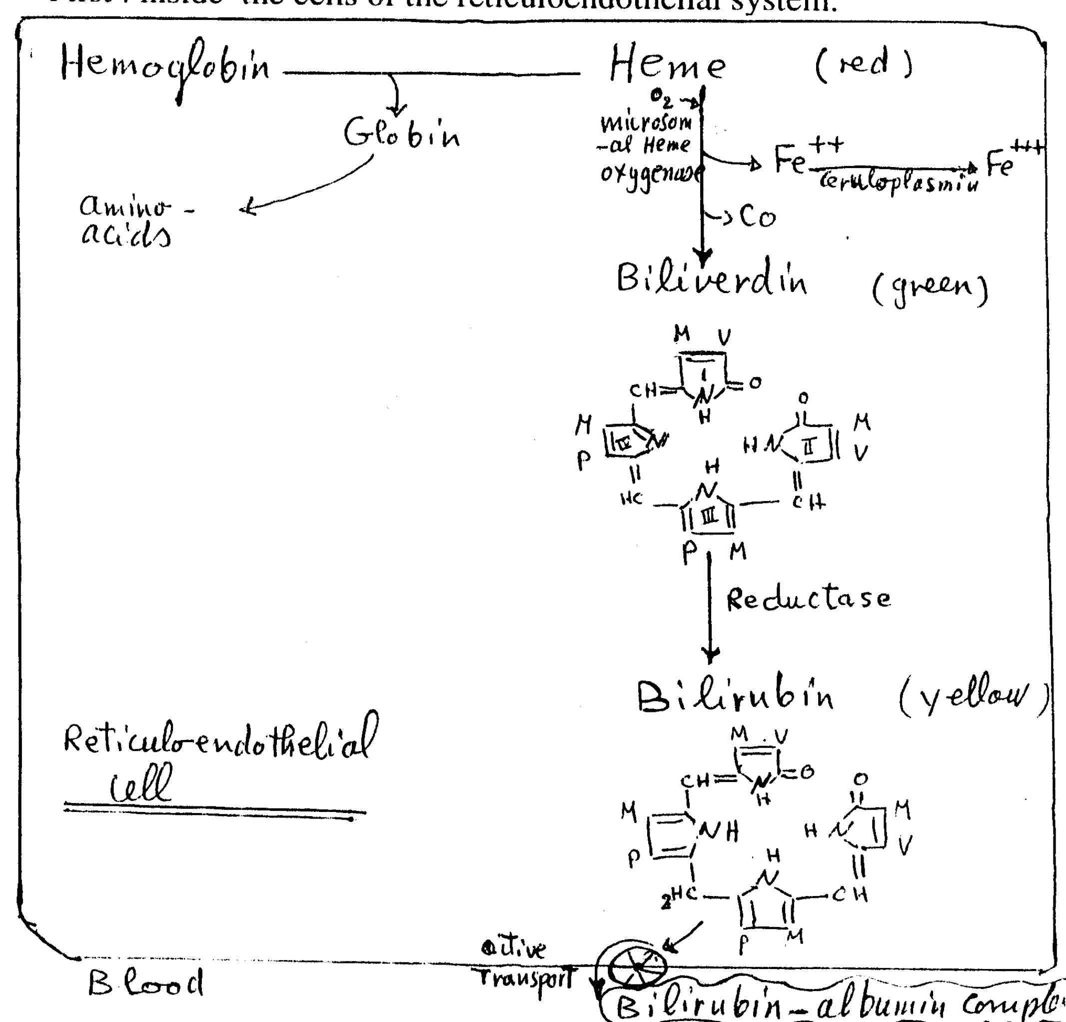
(c) Mixed porphyria " erythropoetic and hepatic ".

The effects of porphyrias are mainly due to increase in the level of δ-ALA and porphobilinogen, these effects include acute attacks of abdominal colics in acute intermittent porphyria, peripheral and central neuropathy and photosensitivity. Treatment of porphyrias depends on inhibition of ALA-synthetase by hematin and on ingestion of glucose and avoidance of drugs that induces ALA-synthase as barbiturates and sulphonamides.

Catabolism of hemoglobin:

The red blood cells "RBC's " live 120 days and become then crenated and abnormal in shape. The old RBC's are removed from the circulation by the reticuloendothelial cells in the spleen, lymph nodes and in the bone marrow. Inside the reticuloendothelial cells (REC), the hemoglobin is broken down into globin and heme. The catabolism of heme is then completed in the REC and then in the liver cells and by the intestinal bacterial enzymes as follows :

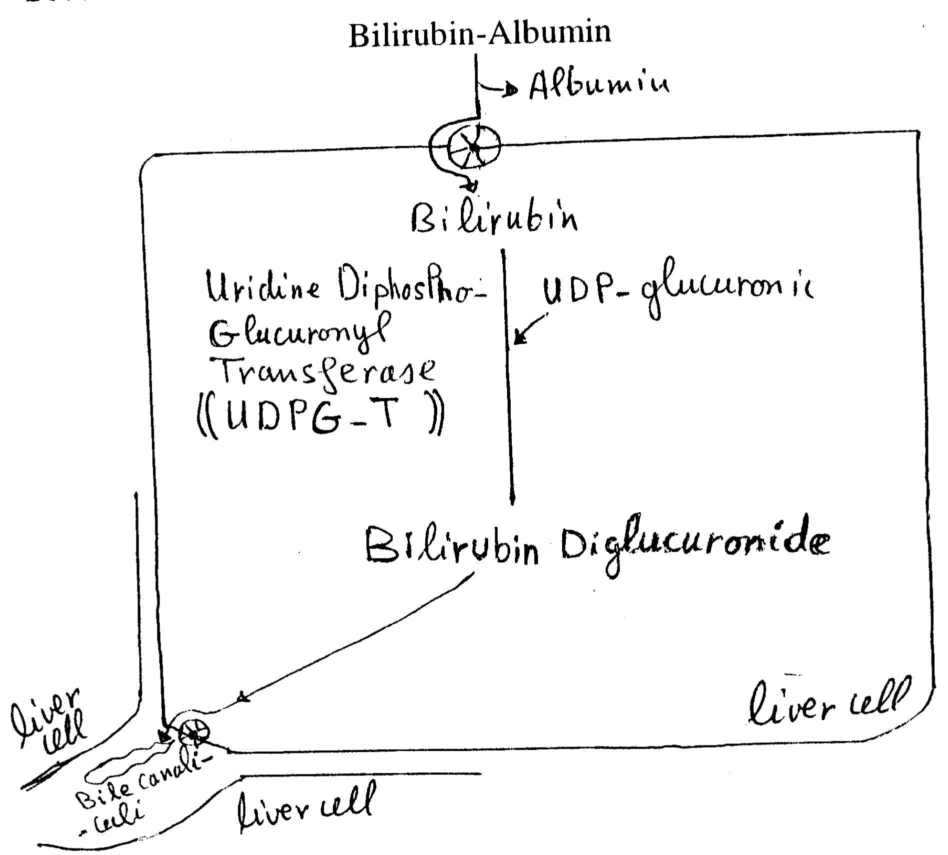
First : inside the cells of the reticuloendothelial system.



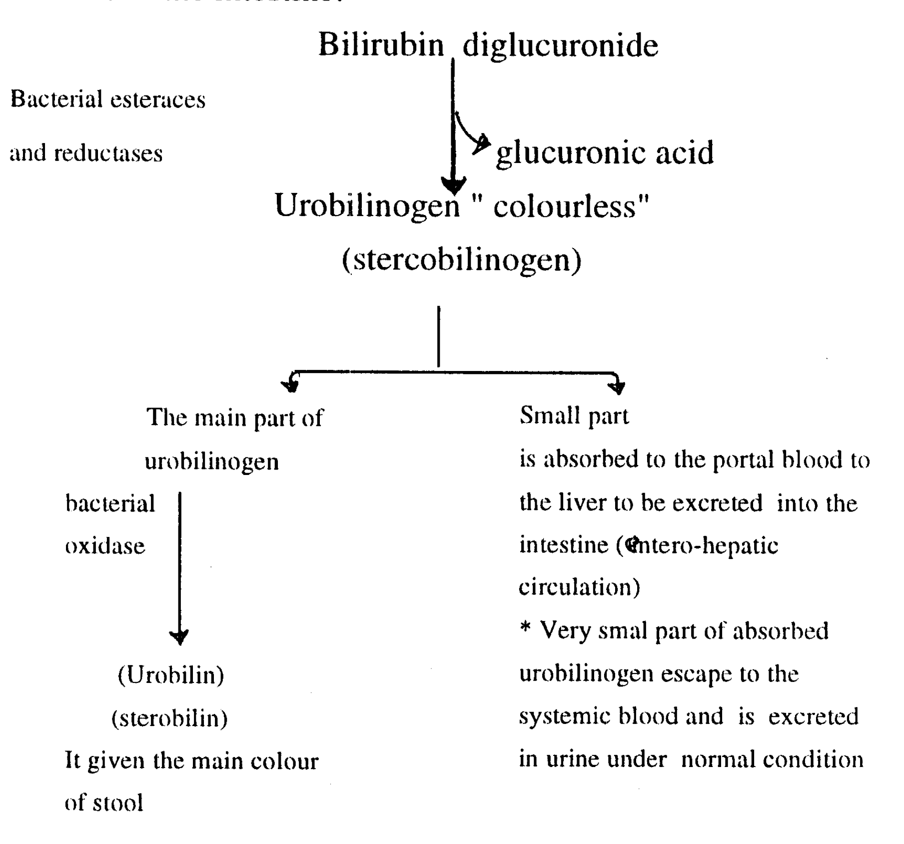
Bilirubin -albumin complex is called:

1. Haembilirubin because it circulates in blood
2. Indirect bilirubin because it does not give a colour directly with diazo reagent. It gives the colour only after addition of ethanol or caffeine in the test tube.
3. Unconjugated bilirubin because it is not conjugated to glucuronic :

Second : Inside a liver cell.



Third : In the intestine:



Cholebilirubin	Haembilirubin
- Bilirubin diglucuronide	- Bilirubin-albumin complex
- Water soluble	- Water insoluble
-Direct reaction with diazo-reagent (Direct bilirubin)	- It gives the colour with diazo reagent after addition of ethanol or caffeive . Indirect bilirubin .
-It can be excreted in urine as in obstructive jaundice	- It can never be present in urine.
-It is conjugated bilirubin because it is conjugated with glucuronic acid	-Unconjugated (it is not conjugated with glucuronic acid).
	-The free bilirubin (without albumin) can pass through cell membranes and become toxic to the cell . It can pass to the brain cells (basal ganglion) leading to damage (Kernicterus). This occurs in sever elevation of Haemebilirubin esp. if albumin is deficient.

Hyprerbilirubinemia - Jaundice

*The normal level of total bilirubin in the blood is 0.1-1 mg/dl. About 1/3 of this level is of the direct type and the other 2/3 are indirect bilirubin.

* When the level of bilirubin in the blood is more than 1 ml /dl, the conduction is called hyperbilirubinaemia.

*If the level of bilirubin in the blood is mor than 2 mg/dl, the bilirubin diffuses into the tissues which then become yellow. This condition is called jaundice or icterus.

The causes of hyperbilirubinaemia include :

(1) Increased production of bilirubin as in haemolytic diseases. The type of bilirubin increased is the indirect " unconjugated" type.

(2) Reduced uptake of bilirubin by the liver cells as in the following conditions.

(a) Drug competition for the carrier. Rifampicin drug competes for the same carrier of bilirubin in the liver cell membrane.

(b) Gilbert disease. Hereditary deficiency of the carrier protein causes mild hyperbilirubinaemia.

The type of increased bilirubin is the indirect .

(3) Reduced conjugation of bilirubin with glucuronic acid in the liver cell as in the following cases :

(a)Neonatal "physiological jaundice".Premature neonates usually have immature UDP-glucuronyl transferase and reduced synthesis of UDP- glucuronic acid. The type of the increased bilirubin is the unconjugated indirect type. Bilirubin can diffuse into the brain especially when its concentration in the plasma exceeds that which can be bound to albumin,this results in kernicterus that may cause mental retardation.

Phototherapy can promote the hepatic excretion of the unconjugated bilirubin in the bile.

Neonatal physiological jaundice is usually a benign condition that terminates spontaneously within 2-3 weeks.

(b) Grigler-Najjar syndrome. It is an inherited deficiency of the conjugating enzyme UDP -glucuronyltransferase in the liver cells . The increased bilirubin is of the unconjugated type. 2 clinical types exist , the type I is severe and type II is mild.

(4) Reduced transport of bilirubin diglucuronide to the bile canaliculi.

Dubin-Johnson syndrome is due to a hereditary defect in the secretion of the conjugated bilirubin from the liver cells to the bile.

(5) Diffuse hepatocellular damage as in :

- Viral hepatitis or in liver cirrhosis.
- Toxic drugs to the liver as chloroform, ether , carbon tetra chloride. The type of bilirubin increased is mainly unconjugated type with some conjugated type.

(6) Obstruction to the flow of bile outside the liver "extrahepatic ".

The most common cause of obstruction is a stone in the common bile duct or cancer head of the pancrease that press on the common bile duct.

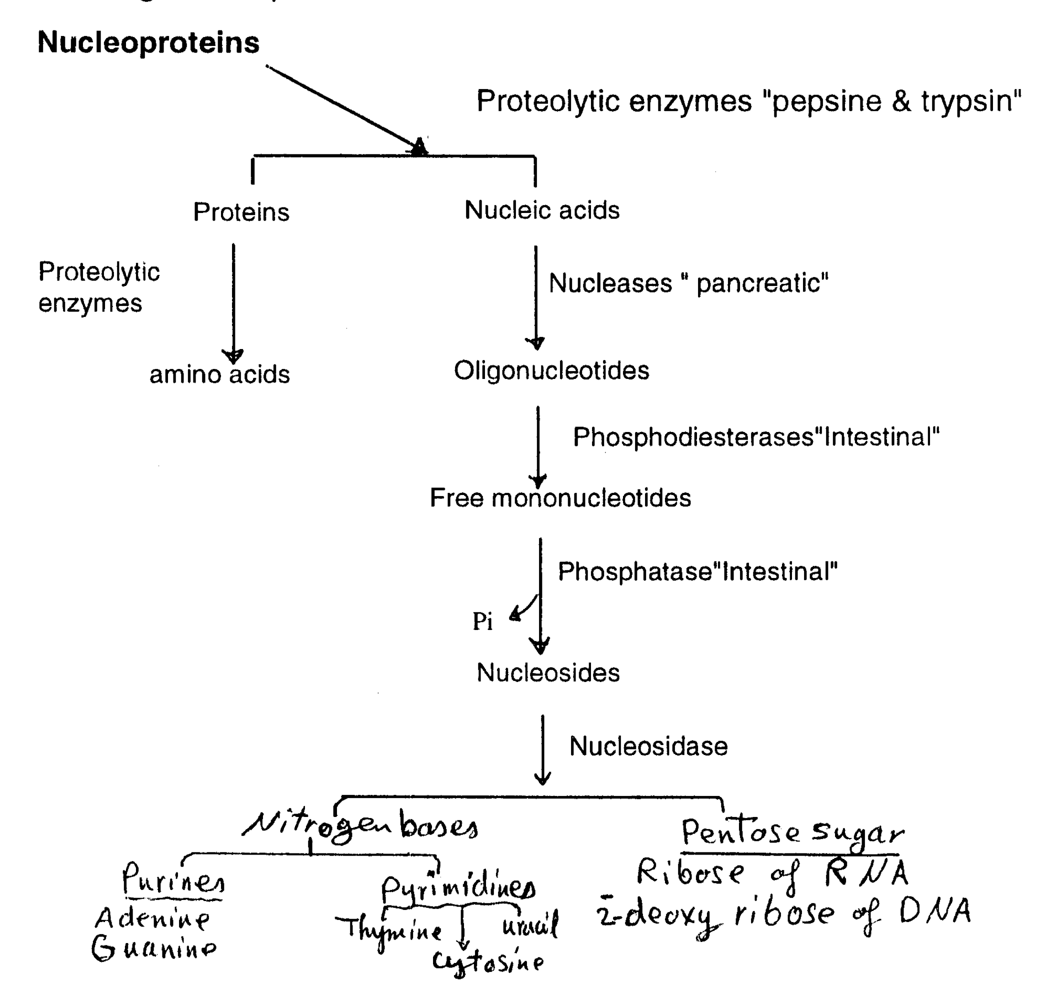
The direct bilirubin is increased in the blood.

NUCLEOPROTEIN METABOLISM

Nucleoproteins are formed of Nucleic acid conjugated with proteins. Nucleoproteins are intracellular proteins.

Digestion of Nucleoproteins:

The protein parts are separated and digested into amino acids by the proteolytic enzymes. The liberated nucleic acids are digested into their original components.



The riboses are absorbed and then excreted in urine. The purine nitrogen bases "adenine and quanines" are oxidized in the intestine by bacterial enzymes into uric acid which is absorbed and then excreted by the kidney into the urine. Therefore, foods that are rich in nucleoprotein as meat, liver and kidney should be restricted in cases of Gout and replaced by non-cellular protein as the milk proteins.

Synthesis of Purine Nucleatides

The nucleotides is formed of nitrogen base + ribose + phosphate. The ribose and the purine nitrogen bases "adenine and quanine" are endogenously formed in most tissues especially in the liver.

- The ribose is synthesized from glucose as ribose – 5 – P by the hexose monophosphate pathway.

The steps of purine nucleotide synthesis can be summarized in two steps as follows :

Step I : Synthesis of phosphoribosyl pyrophosphate (PRPP) by the enzyme PRPP– synthetase.

Step II: The nitrogen and carbon atoms of the purine rings are added on the PRPP molecule from the following precursors namely Aspartic, formic – and methenyl tetrahydrofolate , CO_2 , glycine & Glutamine.

Synthesis of Pyramidine Nucleotides

The primidine nitrogen bases "uracil , cytosine and thymine" are first formed and their precursors are CO_2, glutamine and

asparatic acid. Ribose phosphate is then added from PRPP to form the nucleotides.

Catabolism of Purines:

The purine nitrogen bases "adenine and quanine" present in the nucleotides are continuously catabolised and reformed. The end product of purine catabolism is Uric Acid. Uric acid is excreted by the kidney.

Purine bases "adenine and guanine

↓

Hypoxanthine and xanthine

↓ Xanthine oxidase

Uric acid

Uric acid level in blood is normally 5-7 mg/dl. If the level increases more than the normal level the condition is called Hyperuricaemia which occur in Gout.

Gout: It is a disease caused by increased level of uric acid and deposition of uric acid as urate crystals in the joint, in the kidney and other soft tissues.

- The causes of Gout are :

1- Hereditary increase in the activity of the enzyme PRPP – synthase which leads to increase in the synthesis of purines and hence degradation of purines into urnic acid.

2- Excessive destruction of nucleated cells as in Leukaemia. Prolonged fasting and in severe debilitating diseases.

3- Decreased uric acid excretion as in renal failure. Acidic urine favours deposition of uric acid in urine and formation of uric acid stones.

4- Von–Gierk's disease. Deficiency of enzyme G-6-phosphatase increases G-6-P and increase ribose–5–P which stimulates synthesis of purines.

Treatment of Gout

1- Restriction of proteins of cellular origin as meat, poultry, fish, liver. Restriction of plant xanthines as caffeine in coffee and tea. Milk protein are preferred.

2- Increase intake of fluids to facilitate renal excretion of uric acid.

3- Alkalinisation of the urine to increase the solubility of urates.

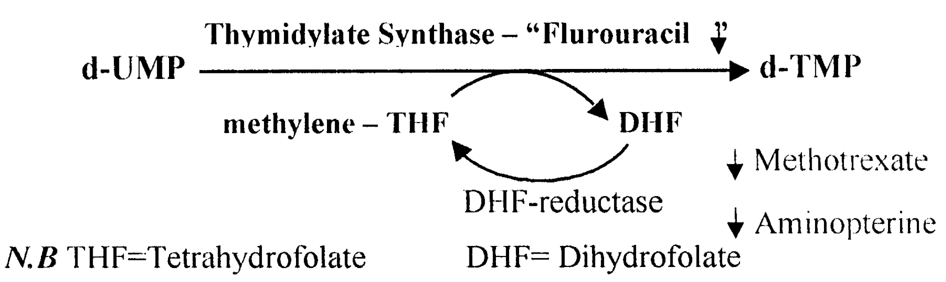
4- A drug called allopurinol "xyloric" inhibits the xanthine oxidase and decreases uric acid synthesis.

Catabolism of pyrimidines

Pyrimidines "Thymine , uracil and cytosine" are catabolized to soluble compounds that can be utilized in metabolism as B-alanine.

Cytotoxic drugs – folic acid antagonists

Several anticancer drugs interfere with folic acid metabolism and inhibits the role of folic acid in the synthesis of the thmidine nucleotide "d-TMP". Thymidine nucleotides are necessary for the synthesis of DNA that occurs during the cell division. Therefore, the folic acid antagonists inhibit the rate of cell division.



N.B THF=Tetrahydrofolate DHF= Dihydrofolate

Malignancy occurs due to uncontrolled cell division which continues to form a mass "Tumour". One line of treatment of malignancy is to inhibit the rate of cell division by drugs, a process called chemotherapy. A group of these drugs inhibit the synthesis of thymidine monophosphate TMP which is required for synthesis of DNA and hence cell division. These drugs include:

1-Flurouracil which inhibits thymidylate synthase

2-Methotrexate which inhibits DHF-reductase

3-Aminopterine which inhibits DHF-reductase

MINERAL METABOLISM

Minerals needed for the body are classified into 2 groups.

1. Minerals needed in high amounts (>100 mg/d). They are called Macrominerals " major minerals" as calcium, Phosphorus, Magnesium, Sodium, Potassium, Chloride .
2. Minerals needed in small " trace" amounts.

They are called trace elements as iodine, zinc, copper,florine, iron, selenium, manganese, cobalt.

CALCIUM AND PHOSPHORUS

Calcium "Ca"	Phosphorus "P"
Distribution in the body:	
Total amounts in 70 kg adult: 1400 g -99% of this total amount is present in Bones and Teeth. -1% in body fluids, 50% of it is present in ionizable form (Ca^{++}), This is the effective calcium form. The other 50% is present in blood bound to albumin protein, it cannot diffuse to cells " non-ionizable". -The bones and teeth contain calcium as hydroxyapatite crystals which is calcium phosphate and calcium hydroxide.	Distribution in the body : The total amount is 700 g. -80% in bone and teeth. -10% in combination with proteins, as nucleoproteins and phosphoproteins and carbohydrates as G-6-(P). -10% in other chemical compounds as coenzymes (NAD,NADP, and ATP, GTP....
Level in blood:	
9-11 mg/dl	2.5 - 5 mg/dl as Inorganic Phosphate (Pi)

Functions : Importance

-Calcium is essential for growth of bones and teeth.

- Calcium decreases neuromuscular excitability.

N.B.: Hypocalcaemia leads to tetany " increase in neuro-muscular excitability and convulsions"

-Calcium is essential for:-

-Normal transmission of nerve impulse.

-Blood clotting

- Muscle contraction

- Calcium activates many enzymes as lipases.

- Some hormone need calcium ions for its activity as Insulin hormone.

Sources:

-Milk and dairy product as cheese and youghurt .

- Egg yolk...

These are the richest sources.

other sources include cabbage

Cauliflower, beans, lentils nuts and figs.

Function :

- Phosphorus is essential for :

1. Formation of bone and teeth
2. Formation of phospholipids that are present in membranes of all cells.
3. Formation of nucleoproteins.
4. Formation of nucleotides present in DNA and RNA, NAD NADP, ATP, COASH, FAD.
5. Formation of surgar phosphates as Glucose-6-phosphate .
6. Formation of phosphprotein as caseinogen of the milk.

Sources :

-Phosphoprotein as caseinogen of the milk .

- Phospholipids and phosphorus in nucleoproteins.

- Other sources as in calcium sources.

Factors Affecting Calcium and Phosphorus Levels in the Blood

Regulation of Ca and P levels in blood.

-Calcium level in the blood should be kept constant at 9-11 mg/dl.

Any variations from this levels causes diseases as in :-

(a) Hypocalcaemia "Ca-level< 9 mg/dl". It cause Tetany " Increased neuromuscular excitability and convulsions.

(b) Hypercalcaemia "Ca-level >11 mg/ dl" causes hypotonia and decreased neuromuscular excitability. Calcification of soft tissues can occur in chronic cases.

The factors that affect calcium level include :

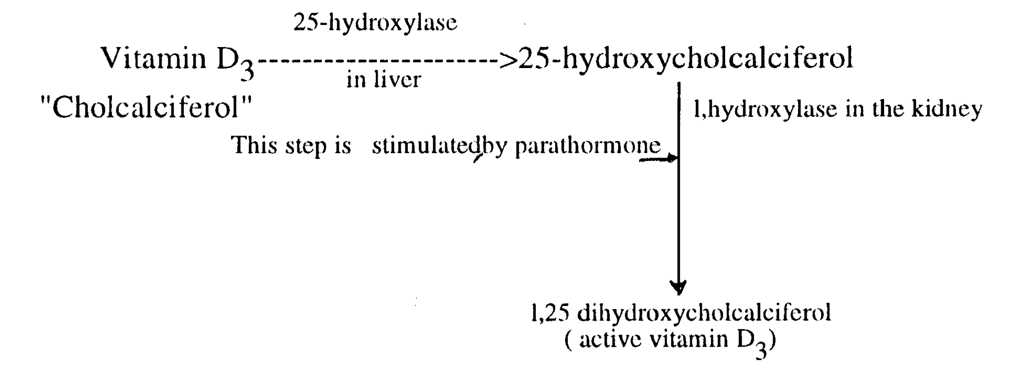
(A) ***Parathyroid hormone***

* It is a peptide hormone. It is secreted from the parathyroid gland. Its secretion is stimulated by low calcium level in the blood.

*Parathyroid hormone = Parathormone .

Causes :

1. Mobilization of calcium from bones to reach the blood.
2. Activation of vitamin D_3 in the kidney.



Active vitamin D_3 stimulates calcium absorption from the intestine. It acts as hormone that induces the synthesis of calcium binding protein.

(B) **Calcitonin hormone.**

* It is a peptide hormone secreted from the thyroid gland. its secretion is stimulated by high level of calcium in the blood.

* Calcitonin causes deposition of calcium in the bone , thus decreassing the high Ca-level in blood.

(C) **Active form of Vitamin D3**

"1,25 dihydroxy cholcalciferol".

* It increase calcium absorption from the intestine. It acts like a hormone.

* It causes deposition of calcium and phosphorus in bones and teeth.

(D) Other factors include :

1. Plasma phosphate.

[Ca] X [P] = Constant.

So increase in P causes decrease in calcium.

2. Acidity and alkalinity in blood. Acidosis increases ionization of calcium Alkalosis decreases ionized calcium.

3. Plasma protein level.

Because 50%of calcium in the blood is bound to albumin any change in albumin level affect the calcium level.

Requirements:

Calcium : 1 g/day for adult to be increased in pregnancy and lactation.

Phosphorus : 1-1.5 g /day.

IRON

Distribution : Total amounts 3-5 g in adult.

* 60-70 % in Hemoglobin as ferrous Fe^{++}

3-5 % in Myoglobin as ferrous Fe^{++}

* 5% in respiratory enzymes " cytochromes ". It acts as electron carrier.

$Fe^{+++} \xrightarrow{e^-} Fe^{++}$.

Ferric Ferrous

* 15-20% stored in liver ,spleen , bone marrow intestine as ferritin. Transferrin is a transport form of iron.

Sources Meat, liver, organ meats, fish , egg yolk,beans, spinach.

Functions = importance

1. Transport of Oxygen from the lungs to the tissues. The iron of hemoglobin (Fe^{++}) carries the oxygen.
2. Store of oxygen in the muscles by ferrous iron in myoglobin.
3. Oxidation in the cell. Iron acts as a prosthetic group in cytochromes, catalases, peroxidases.

Requirements : 10 mg/day for adults to be increased in females (20 mg/day) and infants (15 mg/day) .

Absorption of iron:

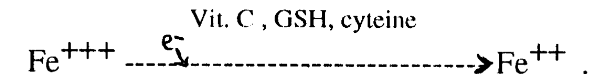
* About 10% of iron taken in food is absorbed.

* Absorption of iron in the intestine is well controlled . It occurs according to the needs " requirements " of the body.

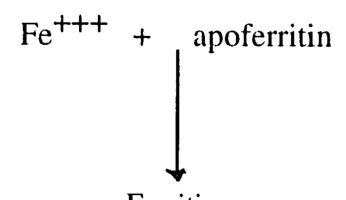
* Only the Ferrous (Fe^{++})form can pass through the membranes.

Mechanism of iron absorption

1. The iron of the food is present as ferric hydroxide $Fe(OH)_3$.
2. HCl of the stomach set the iron of food free as (Fe^{+++}) ferric iron that cannot be absorbed in this form.
3. Ferric iron is then reduced to ferrous by the help of reducing agents as
   - -Vitamin-C "L-ascorbic acid ".
   - - Glutathione " G-SH".
   - - Cysteine amino acid "-SH".



4. the ferrous iron enters through the cell membrane to the inside of the cytoplasm. then it changes to ferric iron and bind to a protein called apoferritin to form ferritin.



5. When the cell is saturated with ferritin the absorption stops.

The iron circulate in the blood bound to a protein called transferin. Normally, 30-40% of the transferrin capacity is utilized in iron transport and 60-70 % of the transferrin remain unbound with iron which is called unsaturated iron binding capacity (UIBC).

Normal levels :

*Plasma iron level in male is 100-140 μg/dl.

* Plasma iron level female is 90-120 μg /dl.

* Total iron binding capacity 300-400 μg/dl.

Diseases related to iron:

(A) Iron deficiency anaemia.

It is mainly caused by chronic blood loss or decrease intake of iron in food.

This type of anaemia is microcytic hypochromic. The TIBC is increased and the plasma iron level is decreased.

(B) Haemochromatosis .

Excessive accumulation of iron in the tissues due to parenteral iron intake or repeated blood transfusions. The iron is deposited in soft tissues leading to their damage. Deposition of iron in the skin causes bronzed colouration. Deposition of iron in the pancrease cause diabetes "Bronzed diabetes Mellitus ".

Iodine (I)

Importance:

The main function of iodine is the synthesis of thyroid hormones with tyrosine amino acid (T_4 and T_3)

-Tyrosine + Iodide ---------> Mono-and diiodotyrosine(MIT & DIT)

- 2 DIT ----------> T_4 (Thyroxine)

-I MIT + DIT ----------->T_3

Sources :

1. See foods
2. In plants grown near the see.

N.b.: Deficiency of iodine was seen in Oasis due to their presence away from the see.

Zinc (Z)

Importance :

1. It is needed for synthesis and storage of Insulin .
2. It is present in certain enzymes as carbonic anhydrase, lactic dehydrogenase (LDH).
3. Zinc is required for mobilization of vitamin A from the liver.
4. Zinc is necessary for normal development of taste buds.

Fluorine :

1. In very small amounts, fluorine prevents dental caries and improves tooth development.

Flourine in very small amounts binds to hydroxyapatite to form fluroapatite.

Flourine + hydroxyapatite (Ca-hydroxide, Ca-phosphate)

↓

Fluroapatite

Fluroapatite resist acids produced by bacterial action on food remnants .

2. Flurine inhibits enolase enzyme in glycolysis .

3. Fluorine inhibits bacterial action on food remnants.

N.B. : Large amounts of fluorine causes mottling of enamel " dental fluoresis".

In this case patchy chalky mottling is produced leading to fragility of teeth.

Requirements : 1-4 mg/day. Drinking water ontain about 1 mg/liter.

Sodium (Na), Potassium (K) & Chloride (cl)

- The sodium chloride is the table salt (Nacl)
- Potassium and sodium are widely distributed in many types of food. Potassium is present in many fruits as citrus fruits and bananas.

Importance:

1- Maintenance of normal fluid distribution between the tissues and the blood. They retain the water due to their osmotic effect. Therefore treatment of hypertension depends on restriction of salt intake to reduce the blood volume and pressure. Most diuretics lead to increase in the urinary loss of Na & K salts.

2- Muscle contraction depends on Na^+ & K^+. sodium ion (Na^+) moves inside and potassium (K^+) moves outside the muscle fibers during contraction. Hyponatremia can cause muscle cramps.

3- Transmission of nerve impluse as a wave of depolarization due to movement of Na^+ to the inside of the nerve fibres.

Excretion: mainly in urine and sweat. The excretion in sweat increases during excessive sweating.

Requirements: 3-5-g/day to be reduced greatly in hypertensive patient.

Tissue Chemistry

Muscle Tissue:

- Muscle represent about 40% of the body weight. The muscle tissue contain (75%) of its weight water, (20%) proteins and (5%) other solids as lipids (3%), glycogen (1%) and inorganic salts.

Muscle Proteins:

(A) Extracellular proteins:

collagen and elastin are present in the connective tissue of the muscle.

(B) Intracellualr proteins:

1-Myosin. It has a globular head and a fibrous tail. The globular head has adenosine triphosphatase (ATP-ase) which hydrolyse ATP to release the energy required for muscle contraction.

2-Actin. It occurs in two forms, G-actin (globular form) and F-actin (fibrous form). The change of the fibrous form to the globular form occurs during muscle contrition and need the energy derived from lysis of ATP by the myosin.

3-Tropomyosin, Troponin and a-actinin. They are associated with actin and help in regulation of muscle contraction.

4-Myogen. It contains all the enzymes of metabolism in the muscles.

5-Myoglobin. It is a chromoprotein that contain iron in the ferrous state (Fe^{++}). It has a red colour. Its molecule contain one polypeptide chain and one heme group. It stores oxygen in the

muscle to be used for muscle contraction when the circulation is partially blocked.

The source of Energy for Muscle Contraction:

1-At the beginning of the reaction, the muscle utilizes the already present ATP which is hydrolyzed by myosin

$$ATP + H_2O \xrightarrow{\text{Myosin}} ADP + Pi + Energy.$$

2- The energy stored in phosphocreatin is utilized to regenerate ATP from ADP

$$\text{creatin - P} + ADP \xrightarrow{CK} \text{creatin} + ATP.$$

N.B CK = creatine Kinase.

3- The accumulated ADP becomes then a source of regenerated ATP by the enzyme myokinase.

$$ADP + ADP \xrightarrow{\text{Myokinase}} ATP + AMP.$$

4-The AMP and released calcium during contraction activate the phosphorylase enzyme which increases glycogenolysis to supply the muscle with glucose –6- phosphate that is oxidized through glycolysis during short duration exercise.

5-If the muscle contractions continues for long period "long exercise", the energy will be derived from oxidation of fatty acids and ketones.



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NOTES ON PRACTICAL BIOCHEMISTRY

By

PROF. ISMAIL HEGAZY



Urine Analysis

urine Analysis (UA) is an important method for assessment of the renal status. The urine analysis includes the physical characteristics, chemical analysis and microscopic examination of the sediments of urine sample.

[A] Physical characteristics:

I. Volume : The normal is 750-2000 ml/day.

2.Odour : Uniniferous

3. Colour : the Normal is amber yellow.

4. Aspect: clear or turbid.

5. Reaction: Acidic pH6.5 (Normal range 5-7).

6. Specific Gravity (SG) : It is defined as the weight of the urine divided by the weight of the water standard (wt. of I000 ml urine/wt. of I000 ml distelled water).

The Normal SG range is I0I5-I025 . It is a measure of the density of the urine which depends on the concentration of the dissolved total solids consisting primarily of NaCl and urea and on the urine volume. S.G. tests the ability of the kidney to concentrate the urine.

The specific Gravity of the urine is measured by the urinometer. It can be also measured by a refractometer " total solid meter".

Procedure :

I. Put 80 ml urine in a cylinder.

2. Place the urinometer in the cylinder and record the reading.

3. Record the room temperatue and calculate.

The specific gravity is then calculated as followsSG= The reading of the urinometer + correction factor for the temperature.

$$SG = \text{the reading} + \left[\frac{\text{R oom Temp. - 15}}{3} \right]$$

[B] <u>Chemical analysis of urine for abnormal constiutents.</u>

The pathological constiutets of the urine which are routinely tested are:

[I] **Albumin** "albuminuria"

The test is heat coagulation test. The test is performed by heating the upper part of a tube filled with urine after filtration. The presence of albumin is detected by coagulation of the protein causing turbidity in the upper portion. The turbidity is increased by addition of few drops of dilute acetic acid.

Proteinuria occurs in glomerulonephritis and pyelonephritis:

[2] Reducing sugars in urine :

Glucose is the main reducing sugar that is detected in the urine in pathological condition as diabetes mellitus. Other reducing sugars that can be present in the urine include lactose in late pregnancy and lactation and in infant's urine. Fructosuria occurs in essential fractosuria, pentosuria occurs the ingestion of large amounts of fruits or fruit juices and also in essential pentosuria.

Detection of glycosuria :

I. Benedict's test :

* 5 ml of Benedict's reagent in a test tube + 8 drops of the urine .
* Boil the mixture for 2 minutes and then cool.
* The blue colour of Benedict's reagent is changed into green, yellow, orange or red indicating the presence of a reducing sugar in the urine

normal urine . The presence of bile salts in urine as in obstructive jaundice will reduce the surface tension and cause the sulphur powder to sink down in the urine sample.

[5] Bilirubin /urobilinogen in the ruine:

The catabolism of hemoglobin results in the formation of the waste product bilirubin which is then conjugated in the liver to bilirubin glucuronide and excreted in the bile to the intestine. Bacterial enzyme in the intestine changes this bilirubin glucuronide to urobilinogen. The majority of this urobilinogen is excreted as the pigment urobilin in the feces. The minority of this urobilinogen is absorbed from the intestine and is excreted by the kidney in the urine "Normally traces of this urobilinogen is present in the urine".

The conjugated bilirubin " bilirubin glucuronide " is water soluble and can appear in urine if the flow of bile is obstructed as in cholestasis " obstructive jaunice". Urobilinogen in urine increases in cases of increased production of bilirubin as in hemolytic jaundice.

Alcoholic Iodine test for bile pigment :

I. 5 ml of urine sample + I0 drops of alcoholic iodine. A green ring gradually develops between the two layers if the urine contains bilirubin glucuronide.

[5] Detection of blood in urine " haematuria"

Red blood cells can be detected by microscopic examination. A chemical test can detect even traces of blood in the urine.

Benzidine test :

I. Prepare a mixture of I0 drops of fresh benzidine solution in glacial acetic acid and I ml of 3% hydrogen peroxide H_2O_2.

2. Tape test for detection of glucose in urine " dipstick .

This test is specific for glucose in urine. The tape contains glucose oxidase and a reduced chromagen " O-toulidine" that chnges its colour in the presence of glucose according to the following reaction

glucose $+O_2 + H_2O$ --glucse oxidase--> gluconic acid $+ H_2O_2$

H_2O_2+ reduced chromagen --peroxidase--> oxidized chromagen(colorued) $+ H_2O$

[3] **Ketone bodies in urine :**

Ketone bodies consist of acetone, aceto-acetic acid ß-hydroxybuteric acid. These substances are products of increased fatty acid oxidiation that occur in cases of uncontrolled diabetes mellitus, low carbohydrate diets and starvation. Ketones are normally not detected in normal urine.Ketones in the urine can be detected by dipstick test depending on Rothera's test.

Rothera's Test :

I. Saturate 5 ml of urine sample with solid ammonium sulphate.

2. Add 5 drops of 5% sodium nitroprusside followed by 2 ml of strong ammonium hydroxide to make the solution alkaline . Deep violet colour appear if ketone bodies are present in urine " ketonuria".

[4] Detection of bile salts.

Sodium tauro-or glycocholate or chenedeoxycholate or their derivatives are excreted in the urine in cases of obstructive liver diseases " obstructive jaundice".

Hay's sulphur test :

Sprinkle a little amount of powdered sulphur on the surface of a urine sample in a test tube. The sulphur floats on the surface of a

2. To this mixture add l ml of urine drop by drop.

A green colour develops if traces of blood exists in the urine .

Microscopic Examination of urine sediments

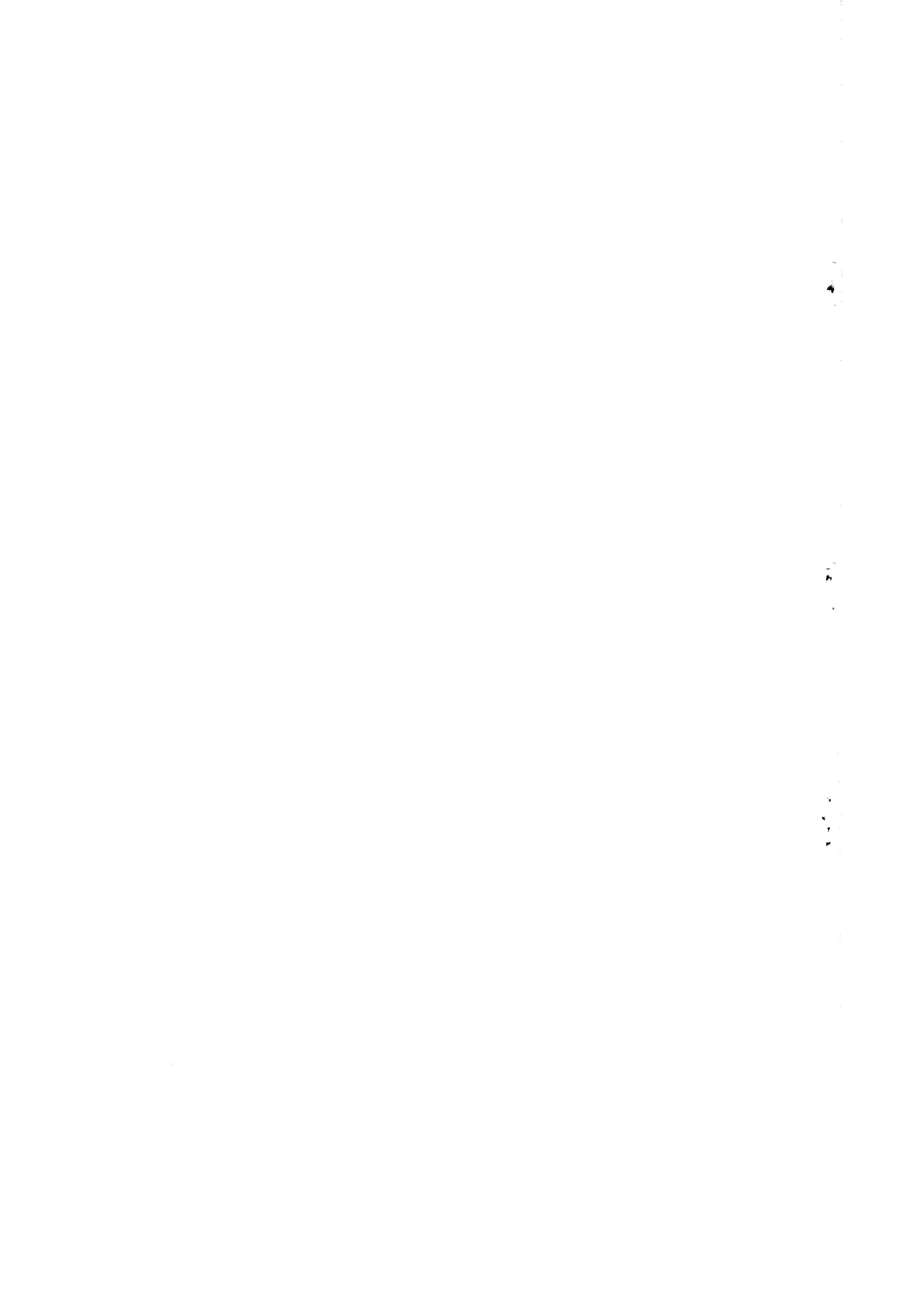
RBC's : Normal count is 0-2 cells/high power field.

WBC's : Normal count is O-l cells/high power field.

Casts : Cast are protein material derived from renal tubules. They appear in renal tubular damage.

Parasitic ova: as schistosoma haematobium.

Crystals : as calcium axalate and amorphus phosphate.



Name : .... **Student's Number :** ....

Sample Number : ....

Report on Urine Sample

(A) Physical Characteristics :

1. Odour :
2. Volume :
3. Colour :
4. Aspect :
5. Reaction:
6. Specific Gravity :

(B) Pathological Constituents:

Test	Observation	Results

The urine sample contains :..